

PHENOLIC EXTRACTION FROM RED HYBRID WINEGRAPES

A Thesis

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ABSTRACT

Cool or cold climate wine regions rely on hybrid winegrapes to produce wines and direct-to-consumer sales to sell wines profitably. Red wine phenolics affect wine quality by contributing to the color, mouthfeel, and ageability of wines. Improving wine techniques that will better extract these compounds can result in higher quality wines. The ability to produce high quality wines is especially important for those regions that rely on hybrid grapes for winemaking. Previous studies investigating the effect of winemaking techniques on phenolic extraction have suggested that exogenous tannin addition can improve color stability and color intensity, as well as increase condensed tannin precursors. However, other studies assessing exogenous tannin addition find no significant differences in the phenolic concentration of finished wines. Furthermore, as most of these studies examine wines produced from *V. vinifera* winegrapes, it is necessary to determine the impact of exogenous tannin addition in wines made from hybrid winegrapes. This study explores the ways commercial tannin addition affect the phenolic concentration of Maréchal Foch, Arandell, and Corot noir wines. Findings suggest that tannin addition timing may affect phenolic concentration; however, commercially recommended dosage may be too low to produce a difference in sensory characteristics finished wines, as previous studies have suggested.

Wine sensory descriptors are used to attract consumers to a particular wine and influence their purchase decision. Consumers perceive wine purchase as a risk, not only because the product is complex, challenging, and intimidating, but also because the sensory experience is the greatest concern. The inclusion of sensory descriptors may reduce risk and help consumers with purchasing decisions, especially when they are unable to sample the wine. In winery tasting rooms, sensory descriptors are often included on tasting sheets to describe a wine's aroma and

flavor to customers. Determining the impact of tasting sheet sensory descriptors on overall tasting room wine sales is important, especially to wineries that rely on direct-to-consumer sales as the primary source of sales, because the majority of their sales are made in the tasting room.

Previous studies in both the food and wine industry show that sensory descriptors increase product sales and consumer appeal. Existing literature, however, focuses on retail settings that may offer a wide selection from many brands and do not, for the most part, allow sampling before purchase. This means that consumers must make choices based on brand recognition and not taste. In a winery tasting room, on the other hand, consumers are encouraged to try many different wines before purchasing. A consumer's decision when supplied with samples, as opposed to just sensory descriptors, may not be the same. There has been no research to determine the effect that sensory descriptors provided with product samples have on consumer choice. Furthermore, there have been no studies investigating the efficacy of sensory descriptors included on tasting sheets. Therefore, we conducted a study in collaboration with nine New York tasting rooms to determine the impact of tasting sheet sensory descriptors on wine sales.

We found that tasting sheets without sensory descriptors increased both bottle and dollar sales, with dollar sales being statistically significant at the ten percent level. Other variables that impacted wine sales included the specific tasting room, the day of the weekend, and festivals occurring in the area.

BIOGRAPHICAL SKETCH

Lauren Thomas is from Richmond, Virginia and graduated from Roanoke College in 2010 with a B.S degree in Biology with Honors. She became interested in wine during a microbiology course during her senior year of college, and started working at a tasting room at James River Cellars Winery after graduation. In 2011, she was accepted to Cornell's Food Science and Technology program to concentrate in enology. Since her arrival to Cornell, she has been involved with a marketing project to examine the effect of tasting sheet sensory descriptors on tasting room sales under the guidance of Dr. Miguel Gómez and Dr. Anna Katharine Mansfield, and she has investigated the extraction of phenolic compounds from red hybrid winegrapes in Dr. Anna Katharine Mansfield's lab.

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CHAPTER 1 LITERATURE REVIEW

1. Phenolics

1.1 Introduction

The United States wine, grape, and grape product industry contributed 162 billion dollars to the American economy in 2007 (MFK Research, LLC 2007). Of this total, national winery sales provided the largest portion at over 11.3 billion dollars. These sales can be attributed to the 4,929 wineries recorded in all fifty states in 2007, an increase of 83 percent since 1999. Represented in this total are the growing wine regions found in the Midwestern and eastern parts of the United States. The majority of these regions are cool or cold climate regions that rely on winter-hardy winegrapes as an alternate to *Vitis vinifera* varieties, as *V. vinifera* cultivars are difficult to grow (Applequist et al. 2008). Progressively, advancements in grapegrowing and winemaking in the Midwestern and eastern United States are leading to better understanding and better quality of hybrid wines.

1.2 Hybrid Grapes

Hybrid grapes are derived from crosses between two different species of grapevines or two cultivars of the same species, and often have three or more species in their ancestry, including European *Vitis vinifera* and American species *Vitis riparia* and *Vitis labrusca* (Pollefeys et al. 2003). Offspring resulting from these crosses are specially developed as cultivars that can survive in cool and cold climate regions due to characteristics such as early ripening, cold hardiness, high productivity, and disease resistance. In the United States, *V. labrusca* varieties account for nearly two thirds of the grapes grown in wine regions in New

York and Washington (Jacobson 2006).

1.2.1 Hybrid Grape Species and Pertinent Cultivars

Most popular winegrapes are *Vitis vinifera*, and may have been cultivated since the Neolithic revolution (Monaghan 2008). These European cultivars include Riesling, Chardonnay, Cabernet Sauvignon, and Merlot. Though *V. vinifera* are found in the United States, they are not native to America. There are, however, several native American vines, including *V. riparia*, *V. rupestris*, *V. muscadina*, *V. rotundifolia*, *V. aestivalis*, and *V. labrusca*. Over time, European and American grapevines have been hybridized to genetically mix sensory characteristics with vigor traits, respectively. These hybrid vines are important to eastern grapegrowing regions, where an assortment of *V. vinifera* and hybrid vines can be found, and are crucial to Upper Midwestern wine regions, where *V. vinifera* die quickly in the subzero climate. Hybrid breeding programs have been established to develop new winegrape varieties to better survive cold and disease pressures, and these programs exist in the United States at universities such as Cornell University and University of Minnesota. The three red hybrid grapes highlighted in this thesis are Maréchal Foch, Corot noir, and the recently introduced cultivar Arandell.

Maréchal Foch

Maréchal Foch is a French-American hybrid grape successfully grown in the United States and Canada. This small, deep purple berry was produced from a cross between Millardet et Grasset 101-14 OP and Goldrieseling, *V. vinifera* (Robinson et al. 2012). This hybrid's complexity comes in part from Millardet et Grasset 101-14 OP, a cross between *V. riparia* and *V. rupestris*. Eugène Kuhlmann made this cross in 1911, and Maréchal Foch was commercialized in 1921 and named after French general Maréchal Ferdinand Foch. In the late 1940s, Maréchal Foch was introduced in Canada, and is currently the most widely planted red variety in Québec.

Maréchal Foch is vigorous, develops tight clusters, ripens early with relatively low acid, and can sustain winter temperatures reaching approximately -35 °C (Robinson et al. 2012). Best suited for cold climates, Maréchal Foch is widely planted in the Midwestern and eastern United States, is particularly popular in Iowa and Illinois, but also found in New York, Wisconsin, Nebraska, Oregon, and other states (Robinson et al. 2012). Wines are usually tannic, and occasionally herbaceous or smoky. This cultivar has low price value, and off-aromas that may be associated with the wine include “beet” and “radish” (Sun et al. 2011).

Corot noir

Corot noir is a hybrid bred at Cornell University and grown primarily in the eastern United States (Robinson et al. 2012). In 1970, Bruce Reisch developed Corot noir from a cross between Seyve-Villard 18-307 and Steuben. Seyve-Villard 18-307 is a cross between Chancellor and Subéreux. Corot noir is a unique and complex hybrid with *V. riparia*, *V. labrusca*, *V. vinifera*, *V. lincecumii*, and *V. rupestris* lineages. It was selected in 1978 as NY70.0809.10 and released in 2006; however, it has been available for testing by growers and research cooperators since 1994 (Reisch et al. 2006).

Corot noir is vigorous, late budding, with deep purple berries that are mid to late ripening and often harvested early to mid October. The vine has moderate winter hardiness and disease resistance (Robinson et al. 2012; Reisch et al. 2006). It is mainly grown in New York, but can also be found in Pennsylvania, Ohio, and Illinois (Robinson et al. 2012). It is produced for both blending or varietal wines, and results in a deep red color wine with cherry and plum fruit aromas and big, soft tannins (Reisch et al. 2006).

Arandell

Created at Cornell University in New York by Bruce Reisch in 1995, Arandell is a cross between two interspecific hybrids NY84.0101.03 and NY88.0514.01 (Cattell 2013). Previously NY 95.0301.01, it was selected in 2001 for propagation and newly released at the Viticulture 2013 Conference in Rochester, NY. Arandell is a mid-season red wine grape that is moderately winter hardy and has a high degree of disease resistance (Reisch et al. 2013). This winegrape produces dark red wines with clean, berry aromas and light to moderate tannins. Wines are characterized with flavors of dark berry fruit and tobacco and hints of black pepper or cedar on the finish, with possibility of vegetal character in cooler years (Cattell 2013).

1.3 Grape and Wine Chemistry

Grapes are considered true berries because they are simple fruits with a pulpy pericarp, or tissue surrounding the seed (Hornsey 2007). The three major tissues of the grape are the flesh (pulp), skin, and seed, all of which contain several compounds important to winemaking. The size of the berry is also important, as smaller berries will have a higher skin to juice ratio (Singleton 1972), and this ratio results in less water dilution, allowing for more concentrated phenolic extraction. Grapes are approximately 75% juice or pulp, 16% skins, 5% stems, and 4% seeds (Margalit 2004), and consist of about water, sugars, organic acids, nitrogenous substances, phenolic substances, inorganic constituents, vitamins, pectins, volatile compounds, and enzymes (Gallander 1974). Phenolic substances are found in the skin, seed, and pulp of grapes, and the two primary phenol groups found in grapes are flavonoids and nonflavonoids (Soleas et al. 1997). The skin contains aromatic compounds, flavor precursors, and 30% of the total berry phenolics, in the form of flavonoids such as flavonols, anthocyanins, and proanthocyanidins (Hornsey 2007). The seeds contain nitrogenous compounds, minerals, and oils, and

approximately 65% of the total berry phenolics in the form of non-flavonoids and flavonoids, largely tannins. The pulp primarily contains hexose sugars (glucose and fructose), organic acids (tartaric and malic), mineral cations, nitrogenous compounds, pectic substances, and 5% of the total berry phenolics as non-flavonoid phenolic compounds (benzoic and cinnamic acids). A study assessing polymeric polyphenols from different grape tissue found that concentrations varied based on cultivar, with 60 to 70% of total extractable phenol content from seeds (a high percentage of this being tannins), 40 to 50% from stems, 23 to 29% from leaves, and 6 to 43% from skins (Kantz and Singleton 1990).

Grape juice contains about 79% water, 20% sugars, 0.6% organic acids, 0.2% inorganic acids, and 0.5% other compounds (Margalit 2004). The juice phenolic compounds are largely non-flavonoids, while the skin and seed phenols are mostly flavonoids and polymers. Alcoholic fermentation converts grape must into wine as yeast cells transform hexose sugars into ethanol and carbon dioxide (Zamora 2009). Concurrently, a number of biochemical, chemical, and physiochemical processes take place. After fermentation, wine consists of water, ethanol, sugars, organic acids, higher alcohols, aldehydes and ketones, esters, nitrogen compounds, inorganic constituents, and phenolics (Soleas et al. 1997). Phenolic compounds are particularly important in wine because they contribute to color stability, organoleptic qualities, and healthful properties. These compounds vary with cultivar, vintage, viticultural practice, and winemaking technique and are related to overall wine quality (Kennedy 2008).

1.3.1 Wine Quality

Grape quality and the maceration period when phenolic extraction occurs contribute to overall wine quality (Kennedy and Peyrot des Gachons 2003). As wine quality is ultimately

based on individual preference, it is much debated and often hard to define. However, an overall opinion is that wine quality refers to ideal sensory attributes offered by the wine (Gawel 2000). High quality relates to an exceptional taste, aroma, and visual appeal for a particular type of wine (Jackson and Lombard 1993; Muñoz et al. 1999) while maintaining a balance of the phenolic compounds extracted (Kennedy and Peyrot des Gachons 2003). There is a correlation between quality ranking and color density in young red wines (Somers and Evans 1974), and higher quality is sometimes observed in wines where more polyphenolic compounds have been extracted (Parenti et al. 2004). Furthermore, phenolic compounds are necessary to support proper wine aging (Díaz-Plaza et al. 2002) and play an important role in creating and maintaining desirable wine color, flavor, and astringency (Kennedy et al. 2005). Anthocyanins in the skins, and flavanol monomers and polymers in skins, seeds, and stems, play an important role in establishing these sensory qualities in wine (Cheynier et al. 2006).

Hybrid wine quality is particularly important, as some believe that there is no quality difference between certain hybrids and *V. vinifera*, while others argue that hybrid wines, particularly of the *V. labrusca* variety, taste different and are poor quality (Unwin 1991). Understanding the important aspects of phenolic compounds in regards to their extraction into wine and complex effects on wine color, flavor, and astringency can help improve hybrid red wine quality.

1.3.2 Wine Phenolic Compounds

Phenolic compounds are extremely important to the color, astringency, bitterness, ageability, and mouthfeel of wines (Kennedy 2008; Margalit 2004; Sacchi et al. 2005). The majority of phenolic compounds found in wine are grape-derived and increase with berry development and maturity, though additional phenolic compounds may come from bacteria or

oak sources. The total phenolic concentration found in the grape is not extracted into the wine, and the majority of phenolic compounds extracted into the wine increases over time with increased maceration time and increased ethanol production (Sacchi et al. 2005). All phenolic compounds contain a phenol aromatic alcohol (Figure 1.3.1) that displays different physical and chemical properties based on the positioning of its delocalized electrons (Margalit 2004). The major phenolic compounds found in wine are hydroxycinnamic acids, anthocyanins, and tannins (Kennedy 2008). However, a number of other phenolic classes exist in wine belonging to the phenolic acid or flavonoid group.

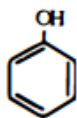


Figure 1.3.1 Phenol alcohol form

Phenolic Acids

The major phenolic acids found in grapes and wine are benzoic acids (Figure 1.3.2) and cinnamic acids (Figure 1.3.3) and their derivatives in the concentration of 100 to 200 mg/L in red wine (Ribéreau-Gayon et al. 2006). These phenolic compounds alone contribute no particular organoleptic properties but may act as precursors to volatile phenols released by yeast and bacteria in wine.

Benzoic acids

Benzoic acids have a C₆-C₁ structure, and are identified by their various R group substituents. These phenolic acids can be found in grapes attached to sugar or esters, and can be hydrolyzed to their free form in red wine. Benzoic acids found in red wine include *p*-hydroxybenzoic acid, protocatechic acid, vanillic acid, gallic acid, and syringic acid with trace amounts of salicylic acid and gentisic acid (Ribéreau-Gayon et al. 2006). Of the benzoic acids,

gallic acid is often found in the highest concentration in wine, as it is found in grapes and can also form during processing from the hydrolysis of hydrolysable and condensed exogenous tannins (Rentzsch et al. 2009).

Cinnamic acids

Cinnamic acids have a C_6-C_3 structure and are differentiated by the varying R group substituents. These compounds can be found in *cis* and *trans* forms, but are often in their *trans* form as it is more stable (Rentzsch et al. 2009). Cinnamic acids are usually found esterified to tartaric acid or sugar. Common free form cinnamic acids found in grapes and wine are *p*-coumaric acid, caffeic acid, ferulic acid, and sinapic acid. Forms found esterified to tartaric acid include coutaric acid, caftaric acid, and fertaric acid (Margalit 2004). In general, the total cinnamic acid concentration averages about 100 mg/L depending on grape variety, location, and grapegrowing practices (Rentzsch et al. 2009). Caftaric acid is the most commonly found cinnamic acid in wine, composing about 50% of the total cinnamic acid concentration. It can react with glutathione in the presence of oxygen during grape and wine processing and form a colorless compound, 2-*S*-glutathionylcaftaric acid or grape reaction product (GRP) (Singleton et al. 1984; Singleton et al. 1985). In a study with Merlot and Cabernet Sauvignon, caftaric acid and coumaric acid concentrations were followed throughout fermentation and aging, and both noticeably decreased during fermentation and slightly during aging (Nagel and Wulf 1979).

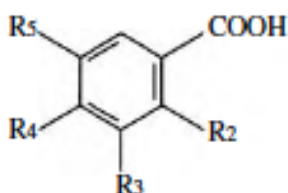


Figure 1.3.2 Benzoic acid structure

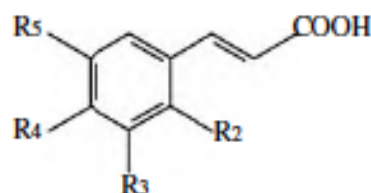


Figure 1.3.3 Cinnamic acid structure

Flavonoids

Flavonoids are a family of phenolic compounds that share a C₆-C₃-C₆ structure (Terrier et al. 2009). This skeleton consists of two phenolic rings (A and B) linked by a pyran ring (Figure 1.3.4). Several classes of flavonoids exist, including flavonols, flavan 3-ols, and anthocyanidins, with different classes distinguished by the oxidation state of the pyran ring. Furthermore, each class is differentiated by changes to the three-ring structure brought about by chemical reactions such as hydroxylation, methylation, glycosylation, acylation, and polymerization (Terrier et al. 2009). The position where each reaction occurs is easily identified by a numbering system (Figure 1.3.4).

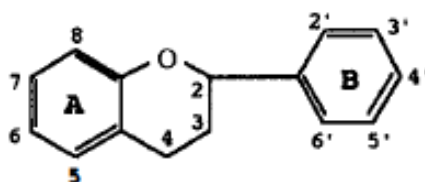


Figure 1.3.4 Flavonoid structure and numbering

Flavonols

The structure of flavonols, including kaempferol, quercetin, and myricetin, is presented in Figure 1.3.5. These compounds can be found in glycoside form in the skins of grapes (Ribéreau-Gayon et al. 2006) and the leaves as they protect the plant from UV rays (Terrier et al. 2009). A study by Price et al. (1995) found that quercetin glycoside and aglycone concentrations increased in grapes and wines with increased sun exposure. Flavonols are hydrolyzed during fermentation and found in their aglycone form in wine in concentrations of approximately 100 mg/L (Ribéreau-Gayon et al. 2006). The concentration depends on the cultivar, stage of development, and climate (Terrier et al. 2009).

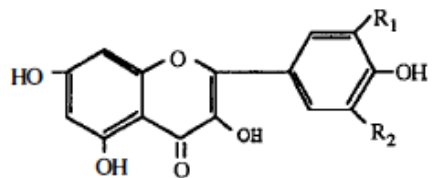


Figure 1.3.5 Flavonol structure

Flavan-3-ols

The structure of flavan-3-ols, or flavanols, is shown in Figure 1.3.6. Flavan-3-ols can be found as monomers, oligomers, and polymers in the seeds, skins, and stems of grapes (Terrier et al. 2009). A study of Merlot grapes found that monomers, oligomers from dimers to decamers, and polymers represented 2%, 8%, and 90%, respectively, of total skin flavanols (Souquet et al. 1996). Flavan-3-ols include isomers (+)-catechin and (-)-epicatechin, which are named based on the conformation of their hydroxyl group and B phenolic ring (Margalit 2004). In grapes, these two compounds are found in fairly equal proportion as monomers (Thorngate 1993). However, monomer concentration in the seed reaches a maximum around veraison but diminishes during ripening as dimeric procyanidin concentration increases. A study assessing the catechin and epicatechin content of various red and white hybrid grape seeds from a vineyard site in the Midwestern United States found ranges of 0.92 to 6.12 mg catechin/g total seed weight and 0.50 to 5.48 mg epicatechin/g total seed weight, suggesting that variation was attributed to cultivar (Applequist et al. 2008). Further, total grape flavan-3-ol concentration varies by cultivar (Thorngate 1993). A study found that must exposed to double or triple the quantity of seeds during fermentation resulted in wines with higher phenolic concentrations, particularly catechin and tannin concentration, than control wines (Kovac et al. 1995).

Flavan-3-ols in grape skins are extracted throughout fermentation, while seed flavan-3-ols are extracted later in fermentation as ethanol content rises (Terrier et al. 2009). An early

study found catechin monomer concentrations increased in Merlot and Cabernet Sauvignon wines until day four of fermentation, then slowly decreased with age (Nagel and Wulf 1979). Epicatechin monomers also decreased in concentration with aging, though it was found in significantly higher amounts in Merlot than Cabernet Sauvignon. A second study suggested that monomers accounted for 38%, 31%, and 47% of total flavan-3-ol concentrations of 76.93 mg/L, 133.18 mg/L, and 89.56 mg/L in Tempranillo, Graciano, and Cabernet Sauvignon wines, respectively (Monagas et al. 2003). When assessing the seeds of these grape cultivars, monomers composed 48%, 57%, and 71% of total flavan-3-ol concentrations of 2.30 mg/g, 7.82 mg/g, and 8.21 mg/g of Tempranillo, Graciano, and Cabernet Sauvignon grape seeds, respectively. Flavan-3-ol concentration in young red wines is approximately 75 mg/L and can reach up to 800 mg/L with age (Thorngate 1993).

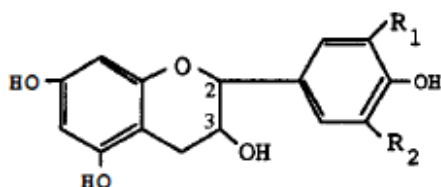


Figure 1.3.6 Flavan-3-ol structure

Tannins

Tannins, or proanthocyanidins, are polymerized flavan-3-ols including catechin, epicatechin, epigallocatechin, and epicatechin gallate, and are located in grape skin and seed tissues (Vivas et al. 2004). Tannins were historically used to tan hides into leather by precipitating proteins; thus tannin refers to this compound's ability to precipitate out proteins (Haslam et al. 1988; Terrier et al. 2009). As the building blocks of tannins, flavan-3-ols link at carbon position 4 of one monomer and position 8 (referred to as C4-C8 linkage) of another, or position 4 and position 6 (C4-C6 linkage), and create branched structures (Terrier et al. 2009).

The C4-C8 or C4-C6 linkages are referred to as B-type proanthocyanidins, or condensed tannins. Proanthocyanidin refers to the fact that when heated in acidic conditions, red anthocyanidin pigments are released. The anthocyanidin released will either be cyanidin or delphinidin, and thus proanthocyanidins are procyanidins or prodelphinidins, respectively. Prodelphinidins contain epigallocatechin units and are only found in the skin, while procyanidins are found in seeds and skin (Souquet et al. 1996). Proanthocyanidins can also be galloylated if the constitutive units are substituted. Their molecular weight varies from 300 to 20,000 g/mol in grapes, with seed tannins having a lower molecular weight distribution than skin tannins (Kennedy and Peyrot des Gachons 2003). Thorngate and Singleton (1994) found that a majority of seed procyanidins are localized in the outer seed coat, while the endosperm contains little polymeric material. A study assessing polyphenolic content of 25 red *V. vinifera* grape seeds cultivated in Greece found an average concentration of 388 mg/100g seed, 49.3% of the total content being catechin (Guendez et al. 2005). Tannin concentration in grapes ranges from 1,000 to 6,000 mg/kg, with one to three times more tannin present in the seed than the skin, with differences based on cultivar, location, vintage, and grapegrowing practices (Guendez et al. 2005). The rate of tannin extraction, particularly from seeds, into wine increases through fermentation (Kennedy and Peyrot des Gachons 2003). Incomplete extraction, adsorption, and precipitation with solids and protein hinder tannin extractability, while increasing ethanol concentration and temperature favor extraction into wine (Thorngate 1993; Sacchi et al. 2005; Singleton and Draper 1964). In a study measuring 1,325 commercial red *V. vinifera* wine cultivars, the average tannin concentration in red wine was 544 mg/L, ranging from 30 mg/L to 1895 mg/L (Harbertson et al. 2008), suggesting that concentration is cultivar dependent.

Mean Degree of Polymerization

The mean degree of polymerization (mDP) reports the average number of polymer subunits (Harbertson et al. 2008). Tannins consist of a first-unit monomer, called the terminal unit, and polymerized extension units. Catechin, epicatechin, epicatechin gallate, and epigallocatechin are major constitutive units of grape skin tannins, with mDP values ranging from three to eighty (Souquet et al. 1996). Catechin, epicatechin, and epicatechin gallate are all terminal units in skin tannin, though catechin is the most abundant terminal unit. Extension units consist of all four monomers, but over 60% are identified as epicatechin (Souquet et al. 1996; Prieur et al. 1994). The mDP found in seed procyanidins ranges from one to sixteen, while the mDP of skin proanthocyanidins averages around thirty (Terrier et al. 2009). Average mDP predicts overall bitterness and astringency of wine (Kennedy and Peyrot des Gachons 2003).

Sensory Impact of Tannins: Bitterness and Astringency

Bitterness and astringency differ in that bitterness is a true taste while astringency is a tactile oral sensation defined as a ‘drying’ or ‘puckering’ (Thorngate 1993). Astringency is considered the most important oral sensation to wine, and arises as tannins precipitate salivary glycoproteins and mucopolysaccharides, leaving an impression of palate dryness. Flavanols and their polymers have the greatest sensory impact on the bitterness and astringency of the finished wine (Sacchi et al. 2005), and a pleasing and complex mouthfeel sensation is dependent on appropriate astringency (Gawel et al. 2001). The low molecular weight flavan-3-ols, those found in seeds, contribute bitterness to the wine (Kennedy and Peyrot des Gachons 2003; Rossi and Singleton 1966), while condensed tannins contribute to astringency (Rossi and Singleton 1966; McRae et al. 2013). This may be because low molecular weight flavan-3-ols are not large enough to bind and precipitate salivary proteins (Thorngate 1993). However, as the degree of

polymerization increases, so do the number of possible binding sites that can precipitate salivary proteins, theoretically increasing astringency. A study assessing purified proanthocyanidin fractions confirmed that degree of polymerization had the greatest effect on distinguishing fractions as astringency attributes increased with an increase in chain length (Vidal et al. 2003). Furthermore, the presence of epigallocatechin units in test fractions tended to lower the sensory perception of 'coarse', emphasizing the importance of skin tannins on mouthfeel sensations.

Exogenous Tannin

One problem relating to maceration in red wine production is the inability to extract all desired tannins from grape skins and seeds during fermentation of some cultivars or wine types (Thorngate 1993). To compensate for this incomplete solubility, commercial tannins are often added to the wine matrix with the intent to increase tannin concentration and create better color stabilization and aging potential. The United States allows tannin addition for clarification purposes (due to their ability to bind protein) and to adjust tannin content, but it cannot be used to add color (Versari et al. 2012). Commercial tannins are natural products that are water or steam extracted from one or several botanical sources, and will differ based on their chemical nature, extraction protocol, and storage time and conditions (Versari et al. 2012). Producer claims for commercial tannins include improved mouthfeel in wine, protein precipitation, and color stabilization. The polyphenolic compounds found in commercial tannin products are described as condensed or hydrolysable, classified by whether they have undergone condensation or can undergo hydrolysis, respectively, and often predicted by their botanical origin (Phillips 2012).

Hydrolysable Tannins

Hydrolysable tannins are defined as gallotannins and ellagitannins that contain a polyhydric alcohol as the basic structural unit, polymerized with gallic acid or hexahydroxydiphenic acid (HHDP) (Vermerris and Nicholson 2006). These compound can easily be hydrolyzed by acids or bases to form gallic or ellagic acid (Versari et al. 2012). Hydrolysable tannins are traditionally extracted from wood during barrel aging of wine (Jordão et al. 2005). For production of commercial tannins, hydrolysable tannins must be extracted from a nutgall or wood source with high tannin content, such as chestnut, oak, or various exotic woods (Versari et al. 2012). These commercial tannins have to contain at least 20 mg/g castalagin equivalent (Versari et al. 2012). Hydrolysable tannins are used to improve the organoleptic quality of wines during aging (Jordão et al. 2005).

Condensed Tannins

Condensed tannins, or proanthocyanidins, are a group of polyhydroxyflavan-3-ol oligomers and polymers linked by carbon-carbon bonds between the flavanol subunits at carbon 4 and carbon 8 (Scofield et al. 2001). Condensed tannins are extracted from grape or the quebracho tree (Versari et al. 2012). Condensed tannins are naturally found in the skin and seed tissue of grapes as proanthocyanidins. Skin extracts tend to have high epigallocatechin concentrations, higher mDP, and lower proportion of galloylated subunits (Souquet et al. 1996). Seed tannins lack epigallocatechin, have more galloylated derivatives (as extension units), lower mDP, and higher monomeric flavan-3-ols. Condensed tannins do not hydrolyze as easily as hydrolysable tannins because of their C₆-C₃-C₆ bonds (Puech et al. 1999). Commercial condensed tannins require more than 10 mg/g catechin equivalents (Versari et al. 2012).

The benefits of commercial tannins are uncertain due to source diversity, and the effects of timing, purity, and dosage are unclear (Makkar et al. 1993). Furthermore, products are not always clearly marked in terms of botanical origin and chemical nature, and commercial tannin labels can be misleading (Obreque-Sl  r et al. 2009). Although the manufacturer suggests specific addition ranges, the exact concentration of exogenous tannin needed to impact wine sensory characteristics is unknown, as is proper timing of additions. There are several studies investigating the aspects of exogenous tannin addition (Parker et al. 2007; Bautista-Ort  n et al. 2005; Main and Morris 2007; Canuti et al. 2012; Harbertson et al. 2012), but definitive guidelines have not been developed to date.

Anthocyanins

Anthocyanins are water-soluble pigments that are largely responsible for providing color to flowers, leaves, fruits, such as grapes, and wines (Timberlake 1980; Kennedy and Peyrot des Gachons 2003). Anthocyanins are the glycosidic form of anthocyanidins, also referred to as aglycones (Figure 1.3.7) (Timberlake 1980). Major aglycones in *vinifera* grapes include cyanidin, peonidin, delphinidin, petunidin, and malvidin (Monagas and Bartolom   2009). However, these compounds are always found in their glycosidic form as anthocyanins because the anthocyanidin form is unstable, and further diversity arises through acylation of the glucose by acetic, *p*-coumaric, and caffeic acids (Cheynier et al. 2006). Malvidin 3-glucoside is the major anthocyanin in *V. vinifera* (Asenstorfer et al. 2003), and occurs when a sugar, usually glucose, binds at carbon position 3 of the aglycone malvidin (Figure 1.3.8). Glucose molecules can also attach at positions 5 and possibly position 7, and these substituents influence the reactivity of the molecule. In grapes, anthocyanins can either be monoglucosides (sugar attached at position 3) or diglucosides (sugars attached at positions 3 and 5). However, *V. vinifera* varieties produce

monoglucosides exclusively, while hybrid grapes produce both monoglucosides and diglucosides. These flavonoids develop in the berry between veraison and harvest, and are found in plant cell vacuoles in the skins. Anthocyanin molecular weight ranges between 450 and 500 g/mol (Kennedy 2008; Kennedy and Peyrot des Gachons 2003), and concentration in grapes can range from 500 to 1,200 mg/kg based on cultivar, maturity, climate, location, and fruit yield (Kennedy and Peyrot des Gachons 2003). Final concentration in a finished wine depends on the original grape concentration as well as the extraction technique (Monagas and Bartolomé 2009).

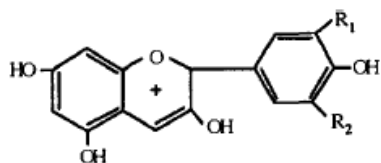


Figure 1.3.7 Anthocyanidin structure

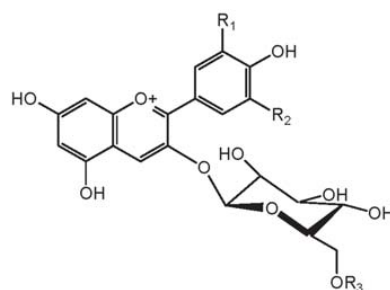


Figure 1.3.8 Anthocyanin structure

Anthocyanins diffuse from the plant cell into the wine matrix during maceration, so that their final concentration in wine is dependent on factors such temperature, cell permeability, surface area over concentration gradient, time, and ethanol concentration (Kennedy 2008). Increasing these factors contributes positively to improved rate of diffusion. Anthocyanin extraction is limited due to the instability of the free form and the difficulty involved in rupturing grape tissue cell membranes to release these compounds (Sacchi et al. 2005). A study evaluating anthocyanin extraction in Cabernet Sauvignon and Tempranillo varieties found that unacylated monoglucosides extract quite rapidly, while acylated forms are extracted more gradually (Mayen et al. 1994). Anthocyanin concentration during fermentation has been shown to increase to a maximum concentration after three days in Merlot and Cabernet Sauvignon, then steadily decline, so that at 240 days post-inoculation less than ten percent of the original concentration

remains (Nagel and Wulf 1979). It is generally agreed that the extraction of anthocyanins peaks during fermentation then decreases (Kennedy and Peyrot des Gachons 2003). This decrease is a result of adsorption on yeast cell walls and precipitation in the form of colloidal material, as well as hydrolysis and condensation reactions with other phenols (Monagas and Bartolomé 2009).

As anthocyanins are chemically unstable and become quite susceptible to transformation in wine (Crus et al. 2010), as the positive charge of the cation can be localized on the oxygen at carbon position 2 or carbon position 4 (Margalit 2004). In an acidic solution, four anthocyanin structures exist in equilibrium: the quinonoidal base (blue), the flavylium cation (red), the carbinol pseudobase or hemiketal (colorless), and the chalcone (colorless) (Brouillard and Delaporte 1977; Monagas and Bartolomé 2009). The flavylium ion is favored at low pH, but as pH increases, the flavylium cation concentration decreases as equilibrium between the quinonoidal form and the hemiketal form, in combination with its chalcone tautomer, occurs (Margalit 2004). The typical wine pH of 3.5 shifts the equilibrium to favor the hemiketal form (Brouillard and Delaporte 1977; Monagas and Bartolomé 2009). Thus, the red color of wine comes primarily from the small proportion (20 to 25 percent of anthocyanins at pH of 3.4 to 3.6) of anthocyanins existing in the flavylium form, and this proportion depends on the pH and SO₂ concentration (Jackson 2008). As an electrophile, the flavylium ion has a partially positive charge at carbon positions 2 and 4, and can attract nucleophilic groups (Margalit 2004). A nucleophilic water or bisulfite ion will form the colorless carbinol or hemiketal. Thus, anthocyanin bleaching can occur when water or bisulfite (from SO₂ addition) reacts with the flavylium ion (Jackson 2008). However, if there is already a substituent in this position, the anthocyanin becomes more stabilized and more resistant to attack by water and SO₂ bleaching (Timberlake 1980). In order to maintain the color of the wine, various reactions occur naturally

during the winemaking process to stabilize the anthocyanin and, thus, protect the wine color. A recent study showed that greater color intensity occurs as the degree of methoxylation increases in the anthocyanin's B-ring (González-Manzano et al. 2008).

Self-Association

One way that the anthocyanin can be stabilized to preserve color, even at higher pH values, is through self-association of the flavylium cation (Timberlake 1980). Self-association increases the color of the wine more than proportionally to the concentration of anthocyanins present (Timberlake 1980). The reaction is driven by hydrophobic interactions that result in a parallel stacking of anthocyanins' aromatic nuclei (Hoshino et al. 1981). This allows the self-associated complex to be surrounded by hydrophilic glucose moieties. For diglucosides, the glucose linked at position 5 plays a greater role in protecting the complex than the 3-glucoside (Hoshino et al. 1980). Self-association results in a hypochromic shift and positive bathochromic shift (Boulton 2001). There is an increase in self-association related to an increase in anthocyanin concentration. A recent study found that malvidin 3-glucoside self-association was thermodynamically favored over copigmentation (Lambert et al. 2011).

Copigmentation

Similar to self-association, copigmentation occurs as stacking between flavylium cation or quinonoidal base chromophores and phenolic compounds, referred to as copigments (Boulton 2001). During stacking, the copigment expels water molecules from the chromophore, protecting it from nucleophilic attack (Brouillard and Dangles 1994). Copigmentation also stabilizes the flavylium cation chromophore preventing color loss (Crus et al. 2010). In young wine, copigmentation accounts for 30 to 50 percent of the color. It is believed that the extent of copigmentation is determined by the quantity of available cofactors (Boulton 2001; González-

Manzano et al. 2008). For common copigments, such as flavan-3-ols, a large copigment-to-pigment ratio is needed for copigmentation to occur, but for good copigments, such as flavonols, the ratio can be closer to one (Brouillard and Dangles 1994). Though considered poor copigments, a recent study found that flavanols contributed significant modifications to wine color when added to model wine containing anthocyanins at concentrations similar to actual wine (González-Manzano et al. 2009). Flavonols, on the other hand, are considered strong copigments, especially quercetin (Lambert et al. 2011). One study suggests that quercetin-to-pigment ratios greater than one can cause the complex to precipitate (Baranac et al. 1997).

Copigmentation is especially important in increasing total anthocyanin extraction the free anthocyanin concentration exists in equilibrium between the skins and wine. As free-form anthocyanin binds to form copigments in the wine, more anthocyanin can diffuse into the wine matrix from grape skins, creating a higher anthocyanin concentration (Boulton 2001). In this way copigmented anthocyanins act as a storage form, allowing more anthocyanins to be extracted and stabilized until polymeric pigments are formed. Wines low in cofactors will not be able to form as many copigmentation complexes, resulting in low concentrations of red pigment. Higher concentration of cofactors results in more copigmentation complexes, and deeper color with blue and purple tones (Boulton 2001).

Copigmentation is similar to self-association in that a positive bathochromatic shift in wavelength occurs, but differs with a hyperchromatic shift in maximum absorption (Boulton 2001). These effects contribute a blue-purple tone to wine, although the actual perceived color depends on the concentration of pigment, ratio of cofactor to pigment, pH, and competing anions in solution. Copigmentation requires a minimum concentration of 18.5 mg/L of pigment, which is easily reached in red wines but not always in rose or blush wines. A study determined that in

aged wines, the amount of copigmented anthocyanin decreased to levels 55% lower than that of the concentration in the initial wine after one year, while polymeric pigment percentage increased (Main and Morris 2007).

Polymeric Color

Unlike copigmentation, polymeric color is a result of anthocyanins bound to condensed tannins, resulting in resistance to decolorization and greater color stability at a broader pH range than copigmented complexes. Anthocyanins quickly complex with polymeric tannins, retaining them in solution. This complex enables long-term color stability for the wine, an increase in degree of polymerization (Singleton and Trousdale 1992; Kennedy 2008; Malien-Aubert et al. 2002), and resistance to sulfur dioxide bleaching (Somers 1971). Polymeric pigment is crucial for proper wine aging and makes up a major portion of color in aged wine. Polymeric complexes begin to form when grapes are crushed and increases steadily over time, comprising 50 to 70 percent of color within the first year and up to 85 percent in older wines (Somers 1971). During formation, anthocyanins can combine with tannins directly or through acetaldehyde-mediated reactions (Salas et al. 2003; Cheynier et al. 2006). Acetaldehyde, a compound naturally produced by yeast cells, provides an intensification of wine color as polymeric pigments increase and anthocyanin monomer content decreases (Somers 1971). This complex will also affect wine astringency, as there is a greater retention of tannin (Singleton and Trousdale 1992). Thorngate and Singleton (1994) propose that the amount of color and tannin in final wines of different cultivars may be due to the presence or absence of an anthocyanin-tannin adduct. This hypothesis is exemplified in Pinot noir, which lacks the adduct; while Pinot noir grapes have high concentrations of tannin, the resultant wines have very low tannin concentrations.

1.4 Influencing Wine Phenolics

Winemaking techniques are intended to improve the extraction and development of phenolic compounds to create a phenolic balance in the finished wine (Kennedy and Peyrot des Gachons 2003). The sensorial journey that consumers experience with wine is a result of vineyard and winery practices that knowledgeable grapegrowers and winemakers control in efforts to manipulate the composition of grapes and wines (Kennedy 2008).

Vineyard practices that influence wine polyphenol content include selecting cultivars known to have high phenolic concentration, choosing best quality grapes, sunny weather, and later harvest (Vinci et al. 2008). A recent study showed that delayed harvest time resulted in a higher concentration of most phenolic acids and flavan-3-ols in musts and wines (Tian et al. 2009). A second study suggested that shoot thinning Maréchal Foch increased berry anthocyanins, but did not contribute to wine anthocyanins, while delayed harvest resulted in increased berry anthocyanins and higher anthocyanins in the finished wine (Sun et al. 2011). A study by Zimman et al. (2002) investigating different maceration techniques on phenolic composition found that fruit composition had a dramatic effect on phenolic composition compared to winemaking treatments, suggesting that fruit quality and composition can overwhelm any winemaking treatment.

However, effective vineyard practices must be followed by efficient winemaking techniques to enable the appropriate phenolic extraction from grapes. Maceration should extract a maximal amount of color while maintaining a balanced concentration of tannins (Kennedy and Peyrot des Gachons 2003). Achieving this ideal extraction depends on the diffusion, adsorption, and reactivity of phenolic compounds, which in turn can depend on several winemaking factors. Increasing alcohol content, SO₂ concentration, and temperature correlate with increasing

anthocyanin and tannin extractability, while increased skin contact time can also improve tannin extractability (Ozmianski et al. 1986).

There are several studies that evaluate the effects of winemaking techniques on wine color. Winemaking techniques that may influence phenolic composition of wine include thermovinification, SO₂ additions, higher fermentation temperatures, cooling or freezing grape, cold soak, prefermentation juice runoff, pectolytic enzymes, pump-overs and punch downs, maceration time, and exogenous tannin addition (Sacchi et al. 2005). Thermovinification requires heating skins between 60 and 70 °C for a short period of time, extracting them in juice, pressing, and cooling before fermentation. This practice damages hypodermal grape cell membranes, releasing anthocyanins while denaturing the browning molecule polyphenol oxidase. Manns et al. (2013) found that hot press treatments across three hybrid cultivars resulted in the greatest extraction of phenolic compounds in musts, but these effects were not seen in finished wines.

In general, increasing SO₂ concentration does not have a large impact on phenolic concentration at normal winemaking temperatures (Sacchi et al. 2005). An early study by Ough and Amerine (1967) found that increased SO₂ concentration in Pinot noir musts resulted in more color extraction at a lower fermentation temperatures. Higher fermentation temperatures result in increased phenolic extraction, because the heat increases the permeability of hypodermal grape tissue cells, but an adequate amount of tannin is needed early on in fermentation to store excess anthocyanins or polymeric color will decrease (Sacchi et al. 2005). Cabernet Sauvignon fermented at 70 °F were found to have more color and less tannin over time than fermentations performed at 80 °F or 53 °F (Ough and Amerine 1967). Gil-Muñoz et al. (1990) assessed the effect on Monastrell wine when grape temperature was decreased before crush and found that temperature only influenced polyphenol extraction during early fermentation and without

permanent color improvement, though hydroxycinnamic derivatives increased during fermentation from cold grapes. Cooling or freezing grapes before crush may burst berry cell membranes and release more phenolic compounds (Sacchi et al. 2005). The effect of cold maceration performed by two cryogens (liquid nitrogen and solid carbon dioxide) on Tuscan Sangiovese grapes resulted in an increased extraction of polyphenolic compounds and higher wine quality, respectively (Parenti et al. 2004).

Cold soak requires that must sit at 10 to 15 °C for several hours or days before fermentation, and is thought to improve wine color with aqueous extraction (Heatherbell et al. 1996). However, as ethanol and increased temperature are known to improve extraction based on previous literature (Sacchi et al. 2005; Terrier et al. 2009), significant improvements in phenolic extraction through cold soak are not likely. Puertas et al. (2009) investigated the effects of cold soak maceration, dry ice maceration, prefermentative juice runoff, delestage, and extended maceration on Tempranillo grapes. They found significant differences in color intensity for every treatment compared to the control, but these differences decreased with aging, and only wines with extended maceration showed high anthocyanin content after four months of aging.

Bautista-Ortín et al. (2007) investigated wine color and stability in Monastrell wines made from running off part of the juice prior to fermentation (*saignée*), and others produced with macerating enzyme or enological tannin additions. Prefermentation juice runoff, or *saignée*, removes juice before fermentation to increase the skin to juice ratio and increase phenolic extraction, mimicking the skin to juice ratio that gives smaller berries better phenolic extraction (Sacchi et al. 2005). A greater volume of juice removed in combination with extended maceration showed significant enhancement in concentration of anthocyanin, tannin, and large polymeric pigment (Harbertson et al. 2009). Enzyme addition may increase anthocyanin

concentration by breaking down skin cell walls and releasing pigment, but enzyme preparation purity is a problem (Sacchi et al. 2005). The wines produced by Bautista-Ortín et al. (2007) from run-off juice had the highest color intensity early on but had low stability, and color decreased dramatically at bottling. Enzyme treated wines showed no significant increase in phenolic concentration from control wines. Enological tannin addition resulted in wines with the highest values of color intensity, anthocyanin content, and tannin content. Interestingly, these researchers did not receive the same results with an earlier study investigating the effect of macerating enzymes and exogenous tannin addition on phenolic content in wines (Bautista-Ortín et al. 2005). In their earlier study, wines with enological tannin added had more monomeric and polymeric tannins than the control after eight months of bottling, but also had a higher percentage of yellow color and negative sensory characteristics, while enzymes improved sensory characteristics.

Maceration time and pump-overs or punch downs are common practices used to enhance phenolic extraction during red wine fermentation. Maceration, or contact between grape solids and the wine matrix, is required to extract phenolics and can be continued after fermentation is complete (Sacchi et al. 2005). Kovac et al. (1992) found that length of maceration time, and a high quantity of solids in contact with must, led to a higher concentration of catechins and tannins in wines. Pump-overs or punch downs distribute the 'cap' that forms as CO₂ pushes grape solids to the top of the fermentation vessel. Solids are mixed into the wine matrix several times a day, either by pushing the cap down or pumping juice out from below the cap and spraying it over the top (Sacchi et al. 2005). A final common, though not entirely understood, winemaking technique is commercial tannin addition, which has been the focus of several recent studies to determine the impact of this practice on finished wines, and arbitrate information about

dosage and timing. However, the exact effect of exogenous tannin addition remains unclear, as different studies present varied results, which is expected due to the large variability among products.

1.4.1 Exogenous Tannin Additions

Studies that attempt to elucidate exogenous tannin addition practices mainly focus on the effect that commercial tannins have on phenolic concentration and color stability. Parker et al. (2007) and Bautista-Ortin et al. (2005) used manufacturer recommended dosages (200 mg/L to Shiraz and 400 mg/L to Monastrell, respectively) and found no improvement in the sensory quality of wine. Furthermore, in finished wines there was no difference in total phenolic concentration between treatments, and, although tannin concentration in Shiraz wines was significantly higher in the post-fermentation tannin addition treatment, after two years there was no significant difference (Parker et al. 2007; Bautista-Ortin et al. 2005). Main and Morris (2007) added exogenous tannin at 200 mg/L but saw no increase in total phenolics; however, browning increased, as did total anthocyanin concentration and percentage of polymeric pigment. Canuti et al. (2012) found that commercial tannin added at 200 mg/L prefermentation had a more significant influence on color than the postfermentation addition treatment. Furthermore, grape seed condensed tannin and gallnut tannin had a greater influence than other commercial tannins, and grapes with high phenolic concentration were less influenced by tannin addition (Canuti et al. 2012). A group who added 200 mg/L of exogenous tannin to wine found that tannin addition depreciated the olfactory characteristics and harmony of the wine (Diaz-Plaza et al. 2002). However, Cíková et al. (2008) analyzed the effect of tannin addition at 10 mg/L and 50 mg/L to several white and rose wines on sensory evaluation and found an overall improvement in sensory attributes in 80 percent of the wines tested at one or both dosages.

A study investigating the effect of three different dosages and timings of condensed tannins and hydrolysable tannins (quebracho and chestnut tannins, respectively) and found that the tannin additions had little influence on phenolic composition of the wine other than increasing tannin concentration in the highest dosage treatment (Manfoi et al. 2010). Neves et al. (2010) studied the effect of two commercial grape seed tannin at two different dosages before and after fermentation on red wine phenolic composition and found that the higher dosage after fermentation increased color intensity in wines low in total polyphenols. Keulder (2005) also found an increase in phenolic concentration from tannin dosages higher than the manufacturer's recommendation (Keulder 2005). Harbertson et al. (2012) added commercial tannin to Merlot during barrel aging at dosages ranging from 60 mg/L to 300 mg/L and to Cabernet Sauvignon wine post-pressing at a recommended rate (200 mg/L) and excessive dosage (800 mg/L). Though tannin concentration significantly increased in the high dosage treatments for Merlot and Cabernet Sauvignon, the recommended dosages were too low to impact total phenolic concentrations, and, although the extreme dosage had a measurable impact in the Cabernet Sauvignon, it negatively impacted sensory attributes including decreased perceived sweetness and viscosity and increased earthy flavors. (Harbertson et al. 2012). Romero-Cascales et al. (2005) propose that an excess of condensed tannins might disrupt the anthocyanin to tannin equilibrium to favor tannin polymerization instead of polymeric pigment formation, which would increase yellow color. Finally, a very recent study by Liu et al. (2013) compared five different oenotannins added to pre-fermented must at recommended rates and found that the condensed tannins improved and stabilized wine color after nine months of bottle aging, favoring grape skin tannins over other sources.

The contradictory results of these studies suggest that there may be more specific information needed to predict the effect of exogenous tannin addition on phenolic concentration and color stability. While some studies declare the recommended dosage too low to notice differences (Parker et al. 2007; Bautista-Ortin et al. 2005; Manfoi et al. 2010), others find sensory results at higher rates unfavorable (Harbertson et al. 2012). With the amount of different commercial tannins available, it is important to determine those products that will result in the most favorable winemaking result.

1.5 Instrumental Analysis of Phenolics

Phenolic analysis is commonly and successfully performed using reverse-phase high-performance liquid chromatography (HPLC). HPLC offers the opportunity to identify hundreds of components at sub-ppm concentrations (Manns and Mansfield 2012). This is highly favored for phenolic analysis in a complex wine matrix, particularly hybrid red musts and wines. Samples are prepared by solid-phase extraction (SPE) to fractionate phenolic compounds. SPE cartridges allow for easy separation of phenolics by first removing unwanted wine components, such as organic acids and sugars. Monomeric compounds are then eluted out of the SPE column into test tubes using acetonitrile and evaporated under nitrogen. The monomeric non-flavonoids are removed from the test tube by rinsing three times with ethyl acetate, filtering, evaporating under nitrogen, resuspending in 20 percent methanol, filtered for HPLC analysis. The fraction remaining in the test tube is composed of anthocyanin monomerics and GRP. This fraction is resuspended in 0.01 N HCl and filtered for HPLC analysis. Finally, the SPE column contains a proanthocyanidin fraction. This fraction is eluted with formic acid and 95 percent methanol and evaporated under nitrogen. After evaporation the proanthocyanidin fraction can be placed in darkness at room temperature to stabilize before the phloroglucinalysis reaction.

Proanthocyanidin analysis can be performed by chromatographic methods either designed to analyze the intact proanthocyanidins or proanthocyanidins following acid-catalyzed cleavage (Kennedy and Jones 2001). Methods retaining intact proanthocyanidins provide number average molecular weight and weight average molecular weight as well as distribution information. As acid-catalyzed cleavage easily break interflavonoid bonds, cleavage methods analyze subunit composition and interflavonoid bond location. By depolymerizing the proanthocyanidin, it is possible to determine specific information about the terminal flavan-3-ol monomers. Furthermore, depolymerization produces electrophilic extension units, which can be easily trapped by nucleophilic reagents.

Phloroglucinol and benzyl mercaptan are the most commonly used nucleophilic trapping reagents (Kennedy and Jones 2001). However, benzyl mercaptan has a fairly unpleasant odor and requires a specialized fume hood. Phloroglucinol is odorless and allows for more selectivity when trapping the extension units (Kennedy and Jones 2001; Hemingway 1989). Furthermore, seven major products, consistent with benzyl mercaptan results, are formed when phloroglucinol is used as a trapping reagent (Kennedy and Jones 2001). Phloroglucinol can, thus, be used as a successful nucleophilic trapping reagent, without special handling requirements, to analyze the flavan-3-ols that polymerize to form proanthocyanidin. The phloroglucinolysis solution can be added to the proanthocyanidin reaction and placed in a 50 °C water bath for 25 minutes for the reaction to occur. After the reaction, sodium acetate is added to fraction and the solution can be filtered for HPLC analysis.

Must and wine phenolic analysis requires two different core-shell column chemistries. Core-shell technology provides increased reproducibility, resolution, and flexibility and decreased analysis time, and can be performed using HPLC (Manns and Mansfield 2012). After

must or wine phenolic material is fractionated, a C₁₈ column is used to analyze monomeric components. A pentafluorophenyl (PFP) column allows for direct injection of a diluted must or wine sample. Differing methods are used on each column for full phenolic analysis. The PFP column determines full range anthocyanins, while the C₁₈ column identifies non-anthocyanin monomerics, anthocyanin monomerics, and condensed tannins monomers by phloroglucinalysis. These methods differ in the wavelengths measured, run time, run pressure, column temperature, injection volume, and flow rate, but both deliver chromatograms that allow the compound to be identified based on its wavelength of maximal absorbance and retention time. The compound concentration can be measured with standard curve information from a commercially available standard, or, for compounds not commercially available, semi-quantitatively using standards similar in chemical nature.

1.6 Rationale

An Australian study declared winemakers the primary market for tannin-related innovations, and suggested six market segments based on commercial tannin utilization (Hill and Kaine 2007). The segments were separated into winemakers with the following end goals: color stabilization, correction of fruit faults, specialized characteristics, risk management, and two groups who did not use exogenous tannins. Overall, winemakers are not completely satisfied with available products, as one winemaker complained that strong, stable color is only delivered from commercial tannins 70 percent of the time. From a marketing perspective, this leaves a market of unsatisfied customers and an opportunity to improve the product.

A collection of previous studies provides ambiguous results regarding the appropriate dosage, timing, and product to best enhance wine. Because of the inconclusive, yet promising, results, exogenous tannin addition still needs to be studied to help winemakers understand proper

practices. To better understand exogenous tannin addition, commercial tannins need to be investigated to determine purity and composition of products, as a previous study showed that enological tannin products contained only 12 to 48 percent tannin (Harbertson et al. 2012). This range makes it difficult to develop application recommendations, as products containing less tannin may need a higher dosage to be noticeable, but, at the same time, the uncertainty of the remaining 52 to 88 percent of the product could result in negative effects.

Furthermore, in the United States many grapegrowing regions rely on hybrid grapes for wine production, because the regional conditions cannot easily sustain *V. vinifera* varieties; thus improving hybrid wine quality is imperative in these regions (Stamp 2010). Hybrid red wines could benefit from specialized winemaking techniques, especially those providing higher tannin concentration. For example, after cluster thinning and shoot thinning Corot noir vines, wine tannin extraction was much lower than *V. vinifera* varieties (5 to 6% tannin extraction), with total tannin concentration ranging from 42 to 64 mg/L (Sun et al. 2012). Little work has been done to assess the impact of exogenous tannin addition on red hybrid wine, particularly as it relates to tannin concentration, color stability, total phenolic concentration, and sensory attributes.

It is crucial for economies that use and require hybrid winegrapes to understand the best enology practices to ensure high quality wine. Overall, there is little work identifying composition of red hybrid winegrapes, practices to increase phenolic extraction, or enological products to supplement low levels of phenolic compounds, such as tannins. It is important to give winemakers in the Midwest and eastern parts of the United States information about relevant cultivars and techniques that can be used to improve the quality of their wines.

2. Marketing

2.1 Introduction

Of the 162 billion dollars spent in the United States on wine, grapes, and grape products, 11.3 billion dollars comes from national winery sales (MFK Research, LLC 2007). In 2011, there were approximately 7,090 wineries found across the fifty states, an increase of 144 percent since 2000 (MFK Research, LLC 2012). The majority of these were small wineries that produce less than 25,000 cases annually (Kenkel et al. 2008) and represent high initial investment, as owners can experience a delay in cash flow until wine sales become profitable. As wineries experience high production costs, it is essential to determine the most efficient practices to expedite and enhance winery profitability. Over 27 million consumers visit wineries and wine trails in a given year, thus, greater knowledge of factors that influence and enhance wine sales will help wineries provide better consumer service and improve profits.

2.2 Wineries

Wineries can be classified into three different categories based on their annual case production: mass-commercial, limited-commercial, and elite or exclusive (Jamerson 2009). Most small wineries in the United States fall into the limited-commercial, or ‘boutique,’ category in that their annual case production is fairly small, approximately 10,000 to 25,000 cases annually, often much less than mass-commercial wineries, but their product is not exclusive, and their tasting room is welcoming. Thousands of boutique wineries were established the United States in the 1990s as wine popularity soared (Vine 1997), and most are family-owned, often multi-generational, businesses (MFK Research, LLC 2007). These wineries generally have high production costs, little to no national market share, and few or no distribution channels, but prefer to remain local and stress direct-to-consumer sales (Zucca, 2010).

2.3 Direct-to-Consumer Sales

Direct distribution is a profitable sales channel used widely by wine producers around the world to enhance the value of a product, develop long-term consumer relationships, and ensure satisfied customers (Gurau and Duquesnois 2008). While direct-to-consumer sales have grown to include internet and wine club shipments, they are traditionally seen as sales made in the tasting room (Teaff et al., 2005; Zucca 2010). These sales are especially important to boutique wineries with a limited annual case production, as wineries that cannot produce enough wine to sell through distributors and wholesalers, or cannot afford to distribute and still make a profit, rely on direct-to-consumer sales to sell full-priced bottles and increase profit margins (Dodd 1995). From 2010 to 2011, direct-to-consumer sales increased by seven percent in North America, with an average of sixty-eight dollars spent at New York wineries per visit (Tinney 2012). As direct-to-consumer sales are essential for boutique winery profitability, it is imperative to investigate all factors influencing its success.

2.4 Tasting Rooms

Tasting rooms provide an venue to familiarize consumers with a winery and distribute winery products in direct-to-consumer sales. A 1990 study of New York wineries accredited 60 percent of wine sales to the tasting room in almost half of the wineries surveyed (Henehan and White 1990), and there was a 6.4 percent increase in tasting room visitors for 2010 to 2011, creating an important opportunity to increase winery profits (Tinney 2012). Tasting rooms often follow a differentiated marketing strategy, selling a variety of wines at a range of prices while only promoting one brand and selling primarily in one location. Tasting rooms can also serve as an opportunity to test new products, identify consumer reactions, and better market wines (Dodd

1995), while creating brand awareness and building brand loyalty. One study determined that the appeal of tasting rooms was the ability to taste before purchasing (Getz et al. 1999).

Tasting Room Experience

The tasting room experience can affect the consumer's impression of the winery as a whole and influence purchase decisions. Critical aspects of this experience include quality of wine, service, wine country appeal, winery appeal, and developmental and marketing factors (Getz et al. 1999). Five factors that proved important to create consumer attachment to tasting rooms include basic customer service, visitor education, brand differentiation, tasting room appearance, and purchase assistance (Olsen and Thach 2010). Furthermore, service, entertainment, and aesthetic aspects like labels and displays strongly influences impression and purchases (Dodd and Gustafson 1997). A study in New Zealand found that 46 percent of visitors who purchased wine on their first visit made a post-visit purchase within six to eight months after their visit, and repeat winery visits and post-visit purchases were more likely for these patrons (Mitchell and Hall 2004).

In general, the tasting room experience is a hybrid of education and entertainment, designed with the intention of increasing wine purchases and attracting repeat customers who will spread positive information about the winery (Dodd 1995). Educational opportunities in the tasting room not only describe the winery's story and build appreciation for wine, but they are also shown to positively influence consumer perception and purchase decisions (Ali-Knight 1999). Consumer interest in wine can be expanded with on-site wine education about health benefits, serving options, and popular food pairings (Wargenau and Che 2006). A study found that consumers were more likely to rate wines as higher quality, and pay more per bottle, after a twenty-five minute tutorial in wines than those with training involving sensory aspects of wine

(LaTour et al. 2011). Finally, a study in southwest Michigan found that on-site wine education sparks an overall interest in wine (Wargenau and Che 2006), which may encourage a return visit.

The intangibles of the tasting room experience, such as service satisfaction, can have a greater impact on wine purchases than actual wine quality (Charters et al. 2009). One study found that the “servicescape,” the tasting room environment where service takes place, had a positive significant effect on response behavior and future purchase intention (Altschwager et al. 2011). Novice wine consumers who feel comfortable in the tasting room are more likely to enjoy the experience, linger longer, return, and offer loyalty (Charters et al. 2009). Experienced visitors, however, look for a more unique experience before providing brand loyalty (Dodd 1993). In either case, once brand loyal, these consumers become repeat customers who spend more on wine and wine accessories than first-time visitors (Dodd 1993). Not only will they spend more when they visit additional times, but they also provide positive word-of-mouth for the winery (Dodd 1993).

Location is another important attribute of the winery experience, as wine region is perceived as an important attribute of the total experience (Bruwer and Alant 2009). A study in South Africa found that almost 75 percent of tasters were visiting because they specifically planned a winery tour, or because they were on vacation in that location. Furthermore, the incorporation of a “winescape” including the presence of vineyards, winemaking activity, and wineries where wine is made, stored, and sold, is suggested to enhance the experience (Telfer 2000).

A study found that tasting room environment influences consumer satisfaction differently and is based on consumer personality; for example, neurotic individuals attach themselves less to exciting brands and more to sincere brands, while extraverts show the opposite behavior (Orth

2008), which may impact consumers preference for particular wineries. When trying to establish a relationship with consumers, desirable regional attributes and emotionally appealing stories of winemakers and production methods foster an emotional connection (Dawson et al. 2011). This personalized experience establishing an emotional connection between the visitor and the winery, their products, and the winery staff is an important aspect of the tasting room experience (Fountain et al. 2008). Creating a positive, memorable experience for consumers is crucial to spread positive word-of-mouth and retain repeat customers.

Finally, a study found that consumers approached wine consumption as an aesthetic experience, similar to appreciating art, in that it provokes a sensory, emotional, and cognitive response, pleasure, an evaluative process, and expressions of personal taste (Charters and Pettigrew 2005). Research confirms the correlation between positive previous experience, repeat visits, product affinity, and brand loyalty (Mitchell and Hall 2004). There is also evidence that a taster's enjoyment of wine can influence immediate purchasing decision (Ho and Gallagher 2005). On the whole, a positive tasting room experience is important not only to establish a customer base, but also to ensure high tasting room sales.

2.5 Consumer Risk

Any sort of purchase decision involves consumer risk. Consumers find choosing a specific wine difficult because of the variety of different sensory characteristics, qualities, and prices offered (Lowengart and Cohen 2006). Wine purchase is considered especially risky because it involves a complex product; many consumers find wine to be a challenging and intimidating product to purchase because of this complexity (Taylor et al., 2008). Furthermore, the taste of wine is seen as the risk of most concern to consumers, increasing the pressure for consumers to make the “right” or “best” choice (Mitchell and Greatorex, 1988). Often, once

consumers discover a new wine, they will continue purchasing from the now-familiar brand (Bruwer and Alant 2009).

Reducing Consumer Risk

One way to reduce consumer risk is provide relevant product information that the consumer can use to make an informed decision. A study found that non-verbal information had significantly higher influence on consumer purchasing decision than verbal information. (Szolnoki et al. 2010). Extrinsic wine attributes, in general, effect consumer choice, by influencing liking and purchase intent (Mueller et al. 2001). A study found that low-involvement consumers made purchases based on packaging, bottle and label aesthetics, and closure material (Barber et al. 2007). These extrinsic factors influence consumers' perceived taste of wine, and when tasting wine before purchase, subsequently influences purchase decision (Szolnoki et al. 2010).

Tasting sheets

Many tasting rooms offer visitors guidance through tasting sheets designed to supply information to help consumers find wines to match their taste preferences. Tasting sheets, or tasting notes, list information about featured tasting room wines. These notes often include sensory descriptors as well as other supplemental information (Bender, 2008), such as awards, food pairings, price, discounts, and wine club membership options (Held, 2012). Shelf information is also important to help consumer choice; in one study, star ratings, numerical ratings, and taste descriptors displayed on shelves were found to influence consumer choice (Lockshin et al. 2001).

Wine descriptions can help consumer choice by identifying the product, its quality, and its value, but tasting is still the best tool for wine selection (Lowengart and Cohen 2006). Tasting

rooms further alleviate the uncertainty of consumer choice, reducing the risk involved in making a decision with an unpredictable outcome (Taylor 1974), by allowing consumers to sample their product before purchasing. Tasting room visitors, particularly first-time visitors, use tastings as risk-reduction strategies and count on the tastings to help them make ultimate purchasing decisions (Bruwer and Alant 2009). In particular, consumers use tastings to represent wine complexity through intrinsic factors based on sensory pleasure and personal enjoyment (Parr et al. 2011). Improving consumer confidence through tastings is important, because a taster's confidence level influences the immediate purchase decision (Ho and Gallagher 2005). Thus, tastings are a known way to improve a consumer's confidence in their purchase decision, likely leading to further purchases.

2.6 Sensory Descriptors

Sensory descriptors are used to shape consumer preference and reduce consumer risk when choosing a new wine. Studies have established that consumers consider simple taste and smell descriptors to be important label information for wine choice (Charters et al., 2000), and find elaborate back-label taste descriptions to be valuable when purchasing wine for a special occasion (Mueller et al., 2010). In restaurant settings, descriptive menu labels have not only been found to increase sales by 27 percent, but also increase customers' liking of the meal and restaurant (Wansink et al., 2001). A second study by Wansink et al. (2005) confirmed that descriptive menu labels resulted in an improved liking of food by customers, who rated dishes as more appealing. Tuorila et al. (1998) found that ratings for unfamiliar foods were enhanced when the products were described with positive information. These studies support the idea that not only sales, but also overall perceived quality of a product, are enhanced by descriptors.

Objective & Subjective Descriptors

Wine sensory descriptors, such as those found on the “Wine Aroma Wheel” (Noble et al., 1984; Noble et al., 1987), supply information about the wine, and can be objective or subjective. Objective sensory descriptors often include analytical wine traits derived from grape growing and winemaking practices (Bender, 2008). These descriptors can be confirmed by scientific analysis. For example, wine can be tested for titratable acidity in a laboratory, and subsequently be described as ‘low’ or ‘high’ based on relative concentration of organic acids. In contrast, subjective descriptors are terms that act metaphorically to describe the wine to a consumer who may not understand the scientific terminology (Bender, 2008) and are determined by a reviewer’s palate.

Subjective descriptors that consumers can relate to will also have more influence on consumer liking than an objective term that they do not understand. For those consumers who may not understand such concepts as residual sugar or acidity as they relate to wine, subjective, or aesthetic, descriptors can act as a bridge to help connect analytical wine traits with more familiar concepts. These descriptors may help create an imaginative experience of a wine that will enhance the consumer’s judgment of its quality regardless of actual analytical traits (Dilworth, 2008). Subjective descriptors can vary widely among tasters; for example, one consumer may describe a wine as having a note of ‘honey,’ while another labels it ‘grapefruit.’

Previous studies have investigated the importance of aesthetic sensory descriptors to wine sales. Krumme (2009) noted that expensive wines tend to be described in terms of their authenticity and fullness of flavor. Aesthetic descriptors used for expensive wines are often more specific, and foods recommended for pairing with these wines tend to be more luxurious

(Krumme, 2009). A second study by Ramirez (2010) presented the idea that longer wine descriptions were associated with higher bottle prices. He found that the use of more subjective descriptors added more monetary value than technical, scientific terms (Ramirez, 2010). Quandt (2007) views these aesthetic descriptors as ambiguous adjectives and phrases that illogically portray wines. He argues that random words could be picked from a list of descriptors and be just as impressive as a professional review (Quandt, 2007). Thus, the descriptors simply sound appealing and may not represent what the consumer will actually taste or smell. This opinion is supported by Hope and Patoine (2009) who argue that the descriptors used by wine critics or sommeliers will be different interpretations from those offered by novices, because critics have developed their brain from wine experience and training in a way that novices have not. Professional sommeliers, for instance, have been found to be better at identifying wine-relevant odors than trainee sommeliers (Zucco and Stevenson 2011). Furthermore, a study found that imprecisely used and misperceived language complicates wine evaluation and, while professionals feel confident communicating this vocabulary with consumers, consumers are skeptical about industry wine claims (Charters and Pettigrew 2006).

Consumer Preference

Certain sensory descriptors can influence consumer choice based solely on consumer preference. For example, sensory characteristics such as sweetness, fresh fruit aromas and sherry-like, reductive aromas were positively and negatively related to consumer choice, respectively (Mueller et al. 2001). Consumers also associate wine preference with a personal, subjective approach based on enjoyment, but judge wine quality objectively (Charters and Pettigrew 2003). Thus, consumers may drink a wine for pleasure that is not necessarily considered high quality.

Wine judgments will vary from person to person, but consumers may be manipulated by the objective and subjective descriptors they read on a tasting sheet (Thomas and Pickering, 2003), thereby agreeing to the presence of specific sensory descriptors in wine based on what they are told to taste or smell. A previous study that confirmed sensory descriptors to be important when purchasing wine also demonstrated that consumers struggled to match these descriptors with corresponding wines (Charters et al., 2000), and untrained consumers have been found incapable of matching wine descriptors with a corresponding wine (d’Hauteville, 2003; Weil, 2007). Though Gawel (1997) has shown that very experienced trained tasters correctly identified wines using vague and abstract terms, and untrained, yet very experienced, tasters correctly identified wines using concrete terms, Lehrer (1975) found that wine tasters who consumed wine at least once per week could not match wines to descriptions. These studies identify a major issue because although very experienced tasters (in this case, fourth year enology undergraduates with training and professionals who tasted at least five wines a week for a year) can correctly identify wines when presented with descriptors, the average consumer consuming wine once a week cannot. Furthermore, novices relied on terms offered by advertising to correctly identify a wine sample (LaTour et al. 2011), reflecting the relative worthlessness descriptors hold for novice consumers. A study by Swahn et al. (2010) investigating the effect of sensory descriptors on apple choice found that consumer preference rankings differed when apples were sampled versus when consumers relied solely on sensory descriptors to make their choice.

While there has been evidence that novices and experts can distinguish red and white wine, but not rose, based on smell alone (Ballester et al. 2009), there are also findings that when given a white wine artificially colored red, consumers list odors that mimic those of red wine

(Morrot 2001) and have trouble discriminating wines masked with color (Parr et al. 2003). These studies show that novice wine drinkers are influenced by intrinsic and extrinsic factors, but will ultimately choose a wine based on personal preference. Those wineries that attempt consumer-driven winemaking by better understanding consumer preferences may not benefit from sensory descriptors (Lesschaeve 2007) because consumers may avoid a wine based on its descriptors or may agree to descriptors while not actually being able to identify them.

2.7 Rationale

Existing literature focuses on retail settings that may offer a wide selection from many brands and do not, for the most part, allow sampling before purchase; subsequently, consumers must make choices based on brand recognition or packaging details rather than sensory evaluation. In such environments, consumers have been shown to evaluate grocery items primarily by extrinsic factors rather than intrinsic factors (Richardson et al. 1994). A consumer's decision when supplied with samples, as opposed to just sensory descriptors, may not be the same (Swahn et al., 2010). Tasting rooms, on the other hand, typically allow visitors to sample a limited selection of one brand; drivers of consumer preference and purchase in such an environment is are still under investigation.

There is also disagreement about the reliability of sensory descriptors (particularly aesthetic descriptors), though there is evidence that consumers are influenced by them and use them in purchasing. At present, there is no research assessing the impact of sensory descriptors in the tasting room, or the effect of sensory descriptors on tasting room sales. For boutique wineries reliant on direct-to-consumer sales and improving the tasting room experience to increase profits, it is crucial to understand all aspects that may effect consumer purchase

decisions. Better understanding the role of sensory descriptors in a tasting room setting, and their potential effect on wine sales, will enhance tasting room efficacy and winery profitability.

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CHAPTER 2

THE EFFECT OF TASTING SHEET SENSORY DESCRIPTORS ON TASTING ROOM SALES

Abstract

Purpose: To study the impact that tasting sheet sensory descriptors have on wine sales in tasting rooms that rely on direct-to-consumer sales to sell the majority of their wines, such as those in New York wine regions.

Design/methodology/approach: Nine tasting rooms participated in the study that took place on weekends (Friday, Saturday, and Sunday) during a six-week period in July and August 2012. Tasting rooms alternated tasting sheets by weekend, one including sensory descriptors and one without any sensory descriptors. At the end of each weekend, tasting room managers compiled information on daily wine bottle and (in the case of seven wineries) dollar sales. A multivariate statistical model was created to measure the relationship between the treatment (tasting sheet with or without descriptors) and wine sales, controlling for other variables that could influence wine sales.

Findings: We found that tasting sheets without sensory descriptors increased both bottle and dollar sales, with dollar sales being statistically significant at the ten percent level. Other variables that impacted wine sales included the specific tasting room, the day of the weekend, and festivals occurring in the area.

Originality/value: While there have been studies involving the impact of descriptors on sales of food and wine products, these studies have all taken place in a grocery store or restaurant setting where many different brands and varieties are offered. There has been no research studying the impact of descriptors on wine sales in the tasting room, where tasters have a limited selection and an option to sample products before purchasing. There has also been little research studying aspects of tasting sheets.

Practical implications: Many tasting rooms, particularly in New York, rely on the tasting room for the majority of wine sales. Determining factors that affect sales can help tasting room managers/owners optimize the tasting room experience for maximized profits.

Keywords: Sensory descriptors, tasting room, wine sales, New York wine region

Article Classification: Research paper

Introduction

Wine sensory descriptors are used to attract consumers to a particular wine and influence their purchase decision. Consumers perceive wine purchase as a risk, not only because the product is complex, challenging, and intimidating, but also because the sensory experience is the greatest concern. The inclusion of sensory descriptors may reduce risk and help consumers with purchasing decisions, especially when they are unable to sample the wine. In winery tasting rooms, sensory descriptors are often included on tasting sheets to describe a wine's aroma and flavor to customers. Determining the impact of tasting sheet sensory descriptors on overall tasting room wine sales is important, especially to wineries that rely on tasting rooms as the primary source of sales (such as many in New York), because the majority of their sales are made in the tasting room (Stonebridge Research, 2010).

Previous studies in both the food and wine industry show that sensory descriptors increase product sales and consumer appeal. Existing literature, however, focuses on retail settings that may offer a wide selection from many brands and do not, for the most part, allow sampling before purchase. This means that consumers must make choices based on brand recognition and not taste. In a winery tasting room, on the other hand, consumers are encouraged to try many different wines before purchasing. A consumer's decision when supplied with samples, as opposed to just sensory descriptors, may not be the same, as suggested by Swahn et al. (2010). There has been no research to determine the effect that sensory descriptors provided with product samples have on consumer choice. Furthermore, there have been no studies investigating the efficacy of sensory descriptors included on tasting sheets. Therefore, we conducted a study in collaboration with nine New York tasting rooms to determine the impact of tasting sheet sensory descriptors on wine sales.

Literature Review

Sensory descriptors are used to encourage consumer preference and reduce consumer risk when choosing a new wine. For example, studies have established that consumers consider simple taste and smell descriptors to be important label information for wine choice (Charters et al., 2000), and find elaborate back-label taste descriptions to be valuable when purchasing wine for a special occasion (Mueller et al., 2010). In restaurant settings, descriptive menu labels have not only been found to increase sales by 27 percent, but also increase customers' perceptions of the meal and restaurant (Wansink et al., 2001). A second study by Wansink et al. (2005) confirmed that descriptive menu labels resulted in an improved perception of food by customers, who rated dishes as more appealing. Tuorila et al. (1998) found that ratings for unfamiliar foods were enhanced when the products were described with positive information. These studies support the idea that not only sales, but also overall perceived quality of a product, are enhanced by descriptors. If this holds true with wines, such an effect may have major impacts on smaller wineries, such as those in New York, that have little to no national marketing and rely on direct-to-consumer tasting room sales to return a profit.

Direct-to-Consumer Sales

Direct distribution is a profitable sales channel used widely by wine producers around the world (Gurau and Duquesnois, 2008). While direct-to-consumer sales have grown to include internet and wine club shipments, they are traditionally seen as sales made in the tasting room (Teaff et al., 2005; Zucca, 2010). These sales are especially important to wineries with a smaller annual case production that have little to no national market share, few or no distribution channels, and high production costs (Zucca, 2010). Many New York wineries do not produce enough wine to sell through distributors and wholesalers and still make a profit, so they rely on

direct-to-consumer sales to sell full priced bottles and increase profit margins (Dodd, 1995). Fortunately, from 2010 to 2011, direct-to-consumer sales increased by five percent in New York, with an average of thirty-six dollars spent at New York wineries per visit (Tinney, 2012). One factor influencing this increase in direct-to-consumer sales may be the lessened risk consumers face when purchasing wine from a tasting room versus a retail outlet.

Wine Purchase as a Risk

Wine purchase in a tasting room involves consumer choice and decision-making about a complex product. Many consumers find wine to be a challenging and intimidating product to purchase because of its complexity (Taylor et al., 2008). Furthermore, the taste of wine is seen as the risk of most concern to consumers (Mitchell and Greatedorex, 1988). Tasting rooms alleviate the uncertainty of consumer choice, reducing the risk involved in making a decision based on an unpredictable outcome (Taylor, 1974) by allowing consumers to sample their product before purchasing. Tasting room visitors subsequently use tastings as risk-reduction strategies, and count on the tastings to help them make ultimate purchasing decisions (Bruwer and Alant, 2009). Many tasting rooms provide added guidance to visitors through tasting sheets designed to supply additional information to help consumers find wines to match their taste preferences.

Tasting Sheet Sensory Descriptors

Tasting sheets, or tasting notes, list information about featured tasting room wines. These notes often include sensory descriptors as well as other supplemental information (Bender, 2008), such as awards, food pairings, price, discounts, and wine club membership options (Held, 2012). This study focused on the effect of sensory descriptors, and other supplemental information was not considered.

Sensory descriptors, such as those found on the “Wine Aroma Wheel” (Noble et al., 1984; Noble et al., 1987), supply information about the wine, and can be objective or subjective. Objective sensory descriptors often include analytical wine traits derived from grape growing and winemaking practices (Bender, 2008). These descriptors can be confirmed by scientific analysis. For example, a qualification of “semi-dry” can be confirmed by measuring residual sugar and comparing measurements with industry-defined sweetness scales. In contrast, aesthetic descriptors apply to those subjective terms that act metaphorically to describe the wine to a consumer who may not understand the scientific terminology (Bender, 2008). For those consumers who may not understand such concepts as residual sugar or acidity as they relate to wine, aesthetic descriptors can act as a bridge to help connect analytical wine traits with more familiar concepts. However, aesthetic descriptors are determined by a reviewer’s palate and can vary among tasters. These descriptors may help create an imaginative experience of a wine that will enhance the consumer’s judgment of its quality regardless of actual analytical traits (Dilworth, 2008). For this reason, the function of aesthetic descriptors is debatable.

Previous studies have investigated the importance of aesthetic sensory descriptors to wine sales. Krumme (2009) noted that expensive wines tend to be described in terms of their authenticity and fullness of flavor. Aesthetic descriptors used for expensive wines are often more specific, and foods recommended for pairing with these wines tend to be more luxurious (Krumme, 2009). A second study by Ramirez (2010) presented the idea that longer wine descriptions were associated with higher bottle prices. He found that the use of more subjective descriptors added more monetary value than technical, scientific terms (Ramirez, 2010). Descriptors that consumers can relate to will also have more influence than an objective term that they do not understand. Quandt (2007) views these aesthetic descriptors as ambiguous

adjectives and phrases that illogically portray wines. The argument made is that random words could be picked from a list of descriptors and sound just as convincing to consumers as professional reviews (Quandt, 2007). While these reviews hold much authority among consumers and are used in choosing wines, the descriptors simply sound appealing and may not represent what the consumer will actually taste or smell.

Wine judgments will vary from person to person, but consumers may be manipulated by the objective and subjective descriptors they read on a tasting sheet just as they are influenced by back label wine attributes (Thomas and Pickering, 2003). However, a previous study that confirmed sensory descriptors to be important when purchasing wine also demonstrated that consumers struggled to match these descriptors with corresponding wines (Charters et al., 2000), and untrained consumers have been found incapable of matching wine descriptors with a corresponding wine (d’Hauteville, 2003; Weil, 2007). A study by Swahn et al. (2010) investigating the effect of sensory descriptors on apple choice found that consumer preference rankings differed when they sampled the apples versus when they relied solely on sensory descriptors to make their choice. These findings call into question the necessity of sensory descriptors, especially in tasting rooms.

While there is some disagreement about the reliability of sensory descriptors (particularly aesthetic descriptors), there is evidence that consumers are influenced by them and use them in purchasing decisions. However, most of these studies apply to a grocery store or restaurant setting that would offer many different brands with potentially overwhelming options of styles and varieties. Tasting rooms, on the other hand, typically allow visitors to sample a limited selection of one brand. At present, research assessing the necessity of sensory descriptors in the tasting room, or their effect on tasting room sales, has not been widely reported. The purpose of

this study is to examine the impact of sensory descriptors, both objective and aesthetic, on bottle sales made in the tasting room.

Methods

Tasting room selection

In response to an e-mail proposal sent to all wineries in New York State, seven wineries volunteered the use of nine tasting rooms for the study (two of the participating wineries operated two tasting rooms each). Participating tasting rooms were located in three New York wine regions, including eight in the Finger Lakes, one in Lake Erie, and one in Long Island, and served wineries ranged in size from approximately 4,000 to 20,000 gallons of annual wine production. All participating wineries rely on the tasting room as their primary sales channel.

Modified Tasting Sheet

Participating wineries provided the tasting sheets then in use in their tasting room in electronic media format. These files were edited to create two modified tasting sheets; one sheet listed sensory descriptors for each wine included, and a second sheet omitted wine sensory descriptors. For this study, sensory descriptors included any adjective used to describe the flavor or aroma of the finished wine, both subjective and objective. Awards, pairings, grape varieties used, and viticulture practices noted on the original tasting sheet remained on the modified tasting sheet (see Figure 1 for an example). The modified tasting sheet kept winery logos, fonts, borders, and other aesthetic qualities identical to the original sheet.

[Figure 1]

Pre-study interviews

Initial interviews were conducted with tasting room managers from each participating tasting room to determine clientele demographics, special events taking place at the winery or in

the area, and information about the design and use of tasting sheet. Researchers were specifically interested in learning how sensory descriptors were created and used, and how tasting room attendants present the tasting sheet to customers.

Project design

The study was performed every Friday, Saturday, and Sunday over a six-week period from July 13 until August 18, 2012. Tasting rooms were randomly organized into two groups, one initiating the study with a tasting sheet listing descriptors, and one with the sheet lacking descriptors. Tasting rooms alternated tasting sheets each weekend for the six-week period so that the treated tasting sheet (no descriptors included) was used just as frequently as the non-treated tasting sheet (descriptors included). Total wine bottles sold at the end of each day were recorded by each winery and reported the following week. In some cases, tasting rooms provided their dollar sales information as well. A follow-up interview was conducted with one member of certain tasting room's staff at the end of the study to discuss any differences noticed in customer behavior as tasting sheets were alternated.

Data

Total daily bottle sales data were received from all wineries at the end of each weekend. Seven of the nine tasting rooms provided their total daily dollar sales. Tasting room staff also provided any information on events that may have occurred at the winery. Researchers consulted regional tourism calendars online to record any festivals taking place in the areas surrounding the participating tasting rooms, and obtained weather for each region using online weather information services (Weather Underground, 2012; The Weather Channel, 2012). All sales information was organized in a Microsoft Excel spreadsheet for Fridays, Saturdays and Sundays during the six-week period of the study.

Sales Analysis

Multiple regression analyses of dollar and bottle sales data were conducted to estimate the impact of the treatment (testing sheet with or without descriptors) controlling for other variables that may affect total sales, including the winery, the day of the weekend, special events in the area, and weather conditions. The total sales measures include number of bottles purchased (*Bottles*) and amount of dollars spent (*Dollars*) by consumers on a given day. The estimating equations to examine the link between treatment (no descriptors) and daily sales are:

$$(1) \text{Bottles} = \beta_0 + \beta_1 \text{Treatment} + \beta_2 B + \beta_3 D + \beta_4 E + \beta_5 F + \beta_6 G + \beta_7 H + \beta_8 I + \beta_9 \text{Festival} + \beta_{10} \text{Saturday} + \beta_{11} \text{Sunday} + \beta_{12} \text{Rain} + \varepsilon_{12}$$

$$(2) \text{Dollars} = \alpha_0 + \alpha_1 \text{Treatment} + \alpha_2 D + \alpha_3 E + \alpha_4 F + \alpha_5 G + \alpha_6 I + \alpha_7 \text{Festival} + \alpha_8 \text{Saturday} + \alpha_9 \text{Sunday} + \alpha_{10} \text{Rain} + \varepsilon_{10}$$

where *Treatment* equals one if the tasting sheet has no descriptors and zero otherwise; the variables *B*, *C*, ..., *I*, are dummy variables representing the collaborating wineries; *Festival* equals one if a festival was held in the area on that day and zero otherwise; *Saturday* (*Sunday*) equals one if the day was Saturday (Sunday), zero otherwise; and *Rain* equals one if the day was rainy or mostly cloudy, zero otherwise.

Results

Descriptive statistics of sales performance (dollars and bottles per day) and the corresponding explanatory variables are presented in Table 1. Dollar and bottles sales averaged \$1,860 and 167 per day, respectively. Each tasting room had the same mean and standard deviation of 0.11 and 0.32, respectively, for an equal representation of each winery in the estimating sample. Friday, Saturday, and Sunday also had the same mean of 0.33 and standard deviation of 0.47 for an equal number of observations for each day in the sample. Festivals occurred in the area about a quarter

of the total study period time, resulting in a mean of 0.26 with a standard deviation of 0.44. Rainy and mostly cloudy conditions were experienced about half of the study days, with a mean of 0.53 and a standard deviation of 0.50.

[Table 1]

It is useful to look at simple sales comparisons before presenting results from the multiple regression analysis. Table 2 presents a comparison of mean dollar and bottle sales for selected explanatory variables. Overall, mean dollar sales were \$1,857 without treatment and \$1,863 with treatment, suggesting very little difference in dollar sales with and without treatment. Of the seven tasting rooms providing dollar sales, the highest dollar average was \$2,528 and the lowest was \$515. This result shows that our sample included different tasting rooms of varied sizes. Saturdays brought in the highest amount dollars sales, averaging \$2,603. Festivals decreased winery dollar sales by an average of \$446, and rainy or mostly cloudy conditions decreased dollar sales by an average of \$264. When analyzing bottle sales, descriptive results proved to be very similar. Mean bottle sales exhibited little differences by treatment, as days with descriptors averaged 166 bottles sold and days with no descriptors averaged 169 bottles sold. The average bottles sales within wineries ranged from 36 to 222 bottles. Saturdays averaged the most bottles sold at 212 bottles. Sales were on average 13 bottles less when there was a festival in the area; and sales were two bottles higher during sunny or mostly sunny days. This descriptive analysis is useful, but to assess correctly the effect of treatment on sales it is important to employ the multiple regressions in equations (1) and (2) to control for other factors that may influence daily sales.

[Table 2]

Multiple Regression Results

The parameter estimates from running a regression on equations (1) and (2) are presented in Table 3. The adjusted R-squared value for the bottle regression and dollar regression was 0.52 and 0.63, respectively. These values suggest that approximately 53 percent of variation in total bottle sales and 63 percent of variation in total dollar sales was attributed to the factors included in the model.

The parameter estimates indicate that removing sensory descriptors from the tasting sheet is associated with an average of \$215.53 increase in wine sales in a given day, keeping everything else constant. This parameter estimate is statistically significant at the ten percent level. The bottle regression parameter estimate, for its part, suggests that removing descriptors from the tasting sheet increases wine sales by 14.33 bottles in a given day. However, this result is not statistically significant. The differences in significance may be explained by the fact that in our sample the bottles' sales variable exhibits less variability than the dollar sales variable. These results provide some evidence that, with all other variables held constant, the tasting rooms in our sample may increase sales by removing descriptors from tasting sheets. This result differs from a previous food study that links descriptors with increased sales (Wansink et al., 2001). However, an important difference between this study is the setting, as tasting rooms allow consumers to sample multiple products before purchase, while grocery store and restaurant customers cannot make a final choice based on multiple tastings.

The regression results suggest that variables like tasting room, day of the weekend, festivals, and weather significantly impact wine sales when other variables are held constant. In general, the direction of the impact of these variables is as expected. For most locations, the specific tasting room had a significant effect on total sales at the one percent level of statistical

significance in both dollar and bottle sales. Differences in total wine sales among tasting rooms could be explained by factors such as tasting room size, reputation, and location. Day of the weekend was a main driver of wine sales, showing significance at the one percent level in both the bottle and dollar sales regressions. The parameter estimates suggests that Saturdays were associated with \$1,117.58 increase in dollar sales and 68.47 more bottles purchased, relative to Fridays. Sundays, on the other hand, presented conflicting results, as dollar sales increased by \$253.37 in comparison to Fridays and showed significance at the ten percent level; but bottle sales decreased by 3.14 bottles relative to Fridays although the coefficient is not statistically significant. Festivals negatively affected wine sales in the tasting rooms studied, and are significant at the one and five percent levels in dollar and bottle sales, respectively. Specifically, festivals in the area decreased daily sales by \$543.15 and 29.76 bottles. This may be explained by the fact that a festival creates a central event to attend and draws business away from the tasting rooms. It is important to note, however, that wineries often have a representative booth at festivals, so decreased sales in the tasting room may be balanced by wine sales in the festival. Finally, the coefficient of *Rain* had a positive but statistically insignificant effect on wine daily dollar and bottle sales. This minor difference is likely due to the impact of weather conditions on spontaneous tasting trips, while pre-planned wine tours may be unaffected.

[Table 3]

Conclusions

This study examined the effect of tasting sheet sensory descriptors on tasting room wine sales, focusing on New York tasting rooms that rely mainly on direct-to-consumer sales. As many New York wineries rely on tasting room sales to generate the majority of their revenue, and the generation of sensory descriptors and production of tasting sheets represents time, effort, and

expense, the efficacy of tasting room descriptor sheets on wine sales is of interest. The results suggest that sensory descriptors do not necessarily increase wine sales in the tasting room, in contrast to previous literature examining the effect of descriptors on food and wine products sales in retail outlets such as grocery stores and restaurants. In such outlets, sampling is rarely allowed, so descriptors are the only means consumers have to help make purchasing decisions. In contrast, tasting room visitors are often allowed to sample several wines, perhaps making sensory descriptors less important for decision-making. Complex and unfamiliar sensory descriptors may be intimidating to inexperienced consumers, who may face further frustration if they try a wine based on its sensory description but cannot recognize the same attributes, or if their expectations are not met. Descriptors that are unappealing to certain consumers may deter those who connote the descriptor with a negative sentiment, which result in less liking of the product (Wansink et al., 2000). More experienced wine tasters may have existing sensory expectations based on their knowledge of grape variety or wine style, reducing the effect of sensory descriptors on their choice. In fact, one tasting room involved in the study noted that sensory descriptors seemed to have a greater impact on wines carrying Alcohol and Tobacco Tax and Trade Bureau (TTB) approved fanciful names (in contrast to wines identified by varietal), suggesting that consumers may consult descriptors more when tasting unfamiliar wines. This statement is supported by a study involving apples, which found that when presented with sensory descriptors, panelists favored apples with unfamiliar names which were favored least when identified by name alone (Swahn et al., 2010). This study suggests that consumers may choose an unfamiliar product based on the sensory expectations they create from descriptors. Further work needs to be done to assess the impact of sensory descriptors on tasting choice

versus actual wine purchase and liking in the tasting room, and to determine whether sensory descriptors are more effective with specific wine attributes, such as familiarity.

Though studies show that descriptors helped influence purchase choice in settings offering a large assortment of brands and varieties, they may not be as crucial in a tasting room that offers a limited wine selection. Many tasting sheets give lengthy descriptions of each featured wine, which could be contributing to information overload and poorer purchase decisions (Jacoby et al., 1974). By removing these descriptors, there are fewer terms on the tasting sheet and less information that consumers must process. In a tasting room environment with staff to guide tasters, the extra and often repeated sensory information may not be necessary. The intimate tasting room experience and the idea of tasting room staff as guides may also contribute to the increase in sales without sensory descriptors provided on a tasting sheet. The wine tasting experience, particularly satisfaction with service, has been reported to increase consumer liking and wine purchases (Dodd and Gustafson, 1997). Thus, removing sensory descriptors may allow tasters to become more interactive, resulting in a greater chance of liking and wine purchase.

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Figure 1. Original and modified tasting notes from a Finger Lakes winery.

2011 PINOT GRIS-----NEW RELEASE!!!-----16.00

Deep lakes and sloping shorelines create the perfect climate for growing Pinot Gris, producing rich and fruitful flavors. Dry and full-bodied with decadent flavors of pink grapefruit, honeysuckle and lemon meringue. Enjoy this wine with grilled salmon and fresh herbs or cream-based soups like chowders and vichyssoise.

2011 PINOT GRIS-----NEW RELEASE!!!-----16.00

Deep lakes and sloping shorelines create the perfect climate for growing Pinot Gris. Enjoy this wine with grilled salmon and fresh herbs or cream-based soups like chowders and vichyssoise.

Table 1. Mean and Standard Deviation of Bottle and Dollar Sales Variables

Variable	Description	Mean	Std deviation
Bottle Sales	Total bottle sales per day	167	113
Dollar Sales	Total dollar sales per day	1,860.67	1,029.15
Treatment	0 (Descriptors) or 1 (No Descriptors)	0.49	0.50
Tasting Room A	0 (Tasting Rooms B-I) or 1 (Tasting Room A)	0.11	0.32
Tasting Room B	0 (Tasting Rooms A, C-I) or 1 (Tasting Room B)	0.11	0.32
Tasting Room C	0 (Tasting Rooms A,B, D-I) or 1 (Tasting Room C)	0.11	0.32
Tasting Room D	0 (Tasting Rooms A-C, E-I) or 1 (Tasting Room D)	0.11	0.32
Tasting Room E	0 (Tasting Rooms A-D, F-I) or 1 (Tasting Room E)	0.11	0.32
Tasting Room F	0 (Tasting Rooms A-E, G-I) or 1 (Tasting Room F)	0.11	0.32
Tasting Room G	0 (Tasting Rooms A-F, H, I) or 1 (Tasting Room G)	0.11	0.32
Tasting Room H	0 (Tasting Rooms A-G, I) or 1 (Tasting Room H)	0.11	0.32
Tasting Room I	0 (Tasting Rooms A-H) or 1 (Tasting Room I)	0.11	0.32
Friday	0 (Saturday or Sunday) or 1 (Friday)	0.33	0.47
Saturday	0 (Friday or Sunday) or 1 (Saturday)	0.33	0.47
Sunday	0 (Friday or Saturday) or 1 (Sunday)	0.33	0.47
Festival	0 (No Festival in Area) or 1 (Festival in Area)	0.26	0.44
Rain	0 (Sunny or Partly Cloudy) or 1 (Mostly cloudy or Rainy)	0.53	0.50

Table 2. Mean of Variables Contributing to Dollar and Bottle Sales

Variable	Description	Dollar Mean	Bottle Mean
Dollar Sales	Total dollar sales	1,860.67	-
Bottle Sales	Total bottle sales	-	167
Treatment	0 (Descriptors) or 1 (No Descriptors)	1,857.80 1,863.73	166 169
Tasting Room	Participating tasting rooms		
	A	-	222
	B	-	197
	C	2,291.86	160
	D	1,891.78	132
	E	1,946.73	93
	F	2,046.71	145
	G	515.70	36
	H	2,528.00	204
	I	1,803.92	143
Day of Sale	Friday	1,359.96	146
	Saturday	2,603.97	212
	Sunday	1,618.09	144
Festival	0 (No Festival in Area) or 1 (Festival in Area)	1,988.19 1,541.88	170 157
Rain	0 (Sunny or Partly Cloudy) or 1 (Mostly cloudy or Rainy)	1,999.28 1,734.67	168 166

Table 3. Regression analysis of Total Dollar and Bottle Sales

Variable (Factor)	Dollars	Bottles
Treatment	215.53* (120.64)	14.33 (12.55)
Tasting Room B	-	-110.22*** (23.16)
Tasting Room D	-530.12*** (181.99)	-151.91*** (22.58)
Tasting Room E	-654.47*** (190.62)	-199.13*** (23.36)
Tasting Room F	-375.20** (181.99)	-139.24*** (22.58)
Tasting Room G	-1906.21*** (181.99)	-247.85*** (22.58)
Tasting Room H	-	-79.80*** (22.58)
Tasting Room I	-617.98*** (181.99)	-141.41*** (22.58)
Festival	-543.15*** (137.71)	-29.76** (15.08)
Saturday	1246.44*** (142.32)	68.47*** (15.47)
Sunday	253.37* (137.63)	-3.14 (15.05)
Rain	7.86 (118.90)	6.88 (12.87)
R-Squared	0.63	0.52
No. Observations	126	162

*, **, *** denote statistical significance at the ten, five, and one percent levels, respectively.

CHAPTER 3

PHENOLIC EXTRACTION FROM RED WINEGRAPES: MARECHAL FOCH, ARANDELL, & COROT NOIR

Red wine phenolics affect wine quality by contributing to the color, mouthfeel, and ageability of wines. Improving wine techniques that will better extract these compounds can result in higher quality wines. The ability to produce high quality wines is especially important for cold climate regions that rely on hybrid grapes for winemaking. Previous studies investigating the effect of winemaking techniques on phenolic extraction have suggested that exogenous tannin addition can improve color stability and color intensity, as well as increase condensed tannin precursors. However, other studies assessing exogenous tannin addition find no significant differences in the phenolic concentration of finished wines. Furthermore, as most of these studies examine wines produced from *V. vinifera* winegrapes, it is necessary to determine the impact of exogenous tannin addition in wines made from hybrid winegrapes. This study explores the ways commercial tannin addition affect the phenolic concentration of Maréchal Foch, Arandell, and Corot noir wines. The composition of the enological additive Biotan (*Laffort*, Bordeaux) was analyzed using HPLC, and found to vary from composition reported previously. Findings suggest that tannin addition timing may affect phenolic concentration; however, commercially recommended dosage may be too low to produce a difference in sensory characteristics finished wines, as previous studies have suggested. This work also reports phenolic characterization of Arandell, a newly released hybrid winegrape from Cornell University, for the first time.

3.1 Introduction

The idea of quality as it relates to red wines, especially those produced from hybrid grape cultivars, is difficult to define yet often debated among wine critics. While some believe that there is no quality difference between certain hybrids and *V. vinifera*, others argue that hybrid wines taste different, particularly those with *V. labrusca* ancestry, and lead to poor wines (Unwin 1991). In the United States many grapegrowing regions rely on hybrid grapes to make wine because the regional conditions cannot easily sustain *V. vinifera* varieties; thus improving hybrid wine quality is imperative in these regions (Stamp 2010). Wine quality can refer to ideal sensory attributes (Gawel 2000), and high quality relates to exceptional taste, aroma, and visual appeal for that type of wine (Jackson and Lombard 1993; Muñoz et al. 1999) as well as phenolic balance (Kennedy et al. 2003) contributing to appropriate color, mouthfeel, and ageability (Sacchi et al. 2005). There is a correlation between quality ranking and color density in young red wines (Somers and Evans 1974), and higher quality is observed in wines with more extracted polyphenolic compounds (Parenti et al. 2004). Furthermore, phenolic compounds are necessary to support proper wine aging (Díaz-Plaza et al. 2002) and play an important role in creating and maintaining desirable wine color, flavor, and astringency (Kennedy et al. 2005).

The phenolic compounds in grapes and wine are responsible for color, astringency, bitterness, and other gustatory properties (Margalit 2004; Kennedy et al. 2005). The concentration of final phenolic compounds in the wine is dependent on grapegrowing conditions and winemaking techniques, as well as the cultivar used. During grape development, phenolic concentration is dependent on grape characteristics (Bautista-Ortín et al. 2007) the ratio of berry skin to volume, amount of sunlight exposure, and harvest time (Tian et al. 2009; Vinci et al. 2008) as well as location, vintage, and cultural practices (Kennedy et al. 2003). Winemaking

techniques that affect phenolic concentration during fermentation include maceration time, processing temperature, and enzyme and exogenous tannin additions; however, each compound has a different extraction behavior, and must composition as well as maceration response can be especially variable (Muñoz et al. 1999; Kennedy et al. 2003; Vinci et al. 2008; Zimman et al. 2002; Timberlake and Bridle 1976). Not all of the grape phenolic material is present in the finished wine as a result of fining, incomplete extraction, and phenolic reactions (Kennedy et al. 2003). Because phenolic compounds change the sensory qualities of a wine over time, it is imperative to understand and manage these changes during production and aging (Kennedy et al. 2005).

Phenolic compounds are found in grapes, but become much more pronounced in wines because they are highly soluble in ethanol (Sacchi et al. 2005). Red wines typically undergo maceration during fermentation to extract these phenolic compounds from the grapes, as they are involved with important organoleptic changes (Salas et al. 2004). These compounds contribute to a wine's color and flavor and are subcategorized into nonflavonoids and flavonoids. Of these two classes, nonflavonoids include phenolic acids, such as hydroxybenzoic acids and cinnamic acids, and stilbenes. Found in grapes and wine, these nonflavonoids originate from grape berry pulp and are often esterified with an alcohol, sugar, or, in the case of cinnamic acids, tartaric acid (Kennedy et al. 2005; Margalit 2004). Flavonoids have a unique structure consisting of two aromatic rings connected by a three-carbon chain that is often closed into a ring by oxygen (Margalit 2004). Flavonoids are a major component of phenolic materials in grapes and include anthocyanins, flavan-3-ols, flavonols, and tannins (Kennedy et al. 2005). Anthocyanins, in the skins, and flavanol monomers and polymers, found in skins, seeds, and stems, are important

factors to determine the quality of wine (Cheynier et al. 2006). These compounds affect the color and sensory characteristics, particularly bitterness and astringency, of the finished wine.

In terms of color, anthocyanin concentrations peak during fermentation and then decrease with time (Somers 1966). Anthocyanins can bind with tannins to create polymeric color (Salas et al. 2004) or self-associate or bind to certain phenolic acids and flavonol and flavone subgroups to form copigments (Boulton 2001; Lambert et al. 2011). While copigmented anthocyanins contribute higher pigment concentration with enhanced color in young wines and are pH dependent (Boulton 2001; Cheynier et al. 2006), polymeric pigment creates stable color compounds that are more resistant to pH changes and decolorization, and increase aging potential (Somers 1971). Flavan-3-ols contribute to the sensory characteristics of wine by providing bitterness and astringency. Lower molecular weight flavan-3-ols, from grape seed and stems, contribute bitterness, while higher molecular weight compounds, located in the skin and stems, provide astringency (Cheynier et al. 2006; Vidal et al. 2003; Sun et al. 1999). Greater concentrations of tannin are found in seeds, but are not as easily extracted into wine, leaving skin tannins as the major contributor to wine composition (Cerpa-Calderón and Kennedy 2008). Furthermore, the amount of tannin in the berry is much less than that found in finished wine, and the variability of total tannin concentrations among red wines is thought to be related to winemaking, so tannin additions are commonly used to compensate for low tannin levels or to effect improvements such as color stability and mouthfeel (Harbertson et al. 2008).

Exogenous, or commercial, tannins are polyphenolic compounds and are either condensed or hydrolysable depending on the extraction source (Versari et al. 2013). Condensed tannins, or proanthocyanidins, are flavan-3-ol monomer units extracted from skins and seeds of grapes, while hydrolysable tannins, such as gallo and ellagitannins, often come from a wood or

other plant source (Versari et al. 2013; Canuti et al. 2012). A recent study involving commercial tannin additions found that condensed tannins contributed to redness in wines aged nine months, while hydrolysable tannin had no influence (Liu et al. 2013), and a second study supported the idea that condensed tannin addition stabilizes color and increases color intensity (Canuti et al. 2012). There is also evidence that condensed tannin addition increases catechin and epicatechin concentrations, which could contribute to copigmentation or polymerization into condensed tannins (Álvarez et al. 2009). However, there is literature that found little influence on wine tannin concentration after commercial tannin addition at the recommended dosage, while at high doses there was an increase in tannin concentration but negative sensory evaluations (Harbertson et al. 2012). A second study found no phenolic differences in finished wine with commercial tannins added at the beginning of fermentation, and sensory results that showed lower color and aroma scores for these wines (Bautista-Ortín et al. 2005).

As exogenous tannin addition has shown variable results, but has important potential to improve wine quality and aging, further research is needed to understand the practices that will enhance the finished wine. Furthermore, there is little research done on the impact of tannin addition on hybrid winegrapes, which have lower condensed tannin extractability and tannin concentrations in the wine than do *V. vinifera* varieties (Sun et al. 2012). A study on Cynthiana (*V. aestivalis* spp.) found that condensed tannins added post-fermentation in combination with a macerating enzyme treatment resulted in increased anthocyanins, and polymerization (Main and Morris 2007). This finding is important to the quality of hybrid wines, as hybrid winegrapes contain diglucosides that have a greater color increase associated with copigmentation than glucosides (Boulton 2001). The current study assesses other interspecific hybrid grape varieties

including Maréchal Foch, Arandell, and Corot noir, to determine the effect of exogenous tannin additions on phenolic composition.

3.2 Materials and Methods

3.2.1 Grape Selection and Harvest

Maréchal Foch, Arandell, and Corot noir were sourced from vineyards located in the Finger Lakes region of New York in 2012. Harvest date was determined based on soluble solids, titratable acidity, and pH analyses of randomly sampled grapes, as well as weather conditions and wildlife pressure. Soluble solids assessment in the form of °Brix measurements were performed with a handheld Atago Alpha-PAL refractometer (Bellevue, WA). Titratable acidity was analyzed with a Titrino Plus 848 doser and 869 autosampler (Metrohm USA, Riverview, FL), and pH was analyzed with an Accumet Excel XL25 pH meter (Thermo-Fisher Scientific, Waltham, MA).

Maréchal Foch (Lot 1) was hand harvested from Cornell University Orchards (Ithaca, NY) on September 4, 2012 and stored overnight in a cooler (2° C) before processing. Due to very low yield in lot 1, a second lot of Maréchal Foch (Lot 2) was sourced from a commercial winery in Penn Yan, NY and machine harvested on September 5, 2012. Arandell was hand harvested from Geneva, NY and delivered and processed September 17, 2012. Corot noir was machine harvested by a commercial winery in Romulus, NY and processed on October 11, 2012.

3.2.2 Wine Production

Wines were made in duplicate for each treatment of Maréchal Foch 2, Arandell, and Corot noir. For each duplicate, 21 kg fruit was mechanically crushed and destemmed (Rossi e Cama, Prospero, Pleasantville, NY) and divided into treatment lots. Due to the smaller amount of grapes from Maréchal Foch 1, the control was produced in duplicate while treatments were

produced as singles, and each lot contained 15 kg of fruit. All treatments requiring skin contact were treated with 50mg/L sulfur dioxide. Treatments without skin contact (RASW) were immediately pressed from skins, and 25mg/L sulfur dioxide was added. A Chemwell 2910 multianalyzer with Software Version 6.3 (Awareness Technology, Palm city, FL) was used to measure yeast assimilable nitrogen (YAN) levels by enzymatic analyses (Unitech Scientific, Hawaiian Garden, CA). FermaidK, GoFerm, and diammonium phosphate (DAP) (Lallemand) were added accordingly to reach 200mg/L YAN levels, as recommended by the Scott Laboratories Fermentation Handbook (2011).

All fermentations included a control of 400 ppm Biotan tannin addition (*Laffort*) to must and fermentation on skins, as well as varying tannin timing treatments in which all additions were Biotan at the rate of 400 ppm (Figure 3.2.1). All fermentations lasted for seven days, at which point the amount of residual sugar remaining was measured by Clinitest tablets (Bayer, Etobicoke, ON, Canada); all fermentations reached dryness. Wines remaining on solids were pressed by hand and transferred to three-gallon glass carboys. Wines including a BLEND treatment combined RASW and NO BIO treatments at equal amounts in three-gallon glass carboys, resulting in a Biotan decrease in concentration to 200 ppm. All wines were inoculated with Alpha malolactic bacteria (Lavlin) as instructed by the manufacturer and were analyzed for completion of malolactic fermentation (MLF) by HPLC analysis (Palo Alto, CA).

Maréchal Foch 1 included a control and one treatment with maceration but no tannin addition until MLF (Figure 3.2.1). The NO BIO treatment was split into two lots after fermentation; the first lot received tannin addition before MLF (BIO PRE-MLF), while the second lot received tannin addition after MLF completion (BIO POST-MLF).

Maréchal Foch 2 was divided equally into a control and three treatments including tannin addition during fermentation (after a reduction of 10 °Brix), tannin addition after fermentation, and fermentation without tannin addition until MLF (Figure 3.2.1). All treatments were fermented on the skins for seven days before pressing. The treatment NO BIO was split into two lots after fermentation; the first lot (BIO POST-MLF) received tannin addition after MLF, and the second lot (NO BIO MLF) received no tannin addition. The treatment BIO POST-AF represented a tannin addition treatment before MLF, as MLF commenced immediately after fermentation.

Arandell was divided equally into a control (BIO PRE-AF) and two treatments, consisting of tannin addition to pressed must and fermentation without skins (RASW) and fermentation on skins without tannin addition (NO BIO) (Figure 3.2.1). After fermentation the two treatments (RASW and NO BIO) were blended in equal amounts to create duplicate BLEND treatments.

Corot noir was divided equally into a control (BIO PRE-AF) and four treatments: pressing from solids mid-fermentation and tannin addition (BIO MID-AF), tannin addition after fermentation (BIO POST-AF), tannin addition to pressed must and fermentation without skins (RASW), and fermentation on skins without tannin addition (NO BIO) (Figure 3.2.1). After fermentation RASW and NO BIO were blended at equal ratios to create duplicate BLEND treatments.

Table 3.2.1. Fermentation treatments and treatment codes.

Treatment	Maréchal Foch 1	Maréchal Foch 2	Arandell	Corot noir
BIO PRE-AF (Control)	D	D	D	D
BIO MID-AF	-	D	-	D
BIO POST-AF	-	D	-	D
RASW	-	-	D	D
BIO PRE-MLF	S	-	-	-
BIO POST-MLF	S	D	-	-
NO BIO MLF	-	D	-	-
BLEND	-	-	D	D

BIO PRE-AF (Control): Biotan added at crush, fermented 7 days on skins, pressed from skins, MLF

BIO MID-AF: Grapes crushed, Biotan added after a °Brix drop, finished fermenting (for a total fermentation period of 7 days), MLF

BIO POST-AF: Grapes crushed, fermentation for 7 days on skin, press, Biotan added after press, MLF

RASW (Red as a White): Grapes crushed and pressed from skins, Biotan added, fermented 7 days, blended in 50:50 ratio with NO BIO

BIO PRE-MLF: Grapes crushed, fermented 7 days on skins, pressed from skins, split into lots, Biotan added before MLF (same tannin addition timing as BIO-POST-AF)

BIO POST-MLF: Grapes crushed, fermented 7 days on skins, pressed from skins, split into lots, Biotan added at the end of MLF

NO BIO MLF: Grapes crushed, fermented 7 days on skins, pressed from skins, split into lots, MLF with no Biotan addition

BLEND: Grapes crushed, fermented 7 days on skins, pressed from skins, blended in 50:50 ratio with RASW, MLF

S: Treatment done in singlet

D: Treatment done in duplicate

For all treatments except Maréchal Foch 1, red wines were fermented in 13-gallon stainless steel pots; Maréchal Foch 1 treatments were fermented in 22-quart plastic fermenters. All RASW treatments were fermented in 3-gallon glass carboys. After reaching room temperature, Maréchal Foch (1 and 2) must was inoculated with R2 yeast (*Lalvin*), and Arandell and Corot noir must was inoculated with GRE yeast (*Lalvin*) as instructed by the manufacturer. All musts were kept in a 20°C temperature controlled room, and all fermentations with solids

contact had caps punched down manually twice a day. Once completion of MLF was confirmed by HPLC analysis for organic acids, sulfur dioxide was added to maintain 40mg/L free sulfur dioxide; wines were then cold stabilized at 2°C prior to bottling. After cold stabilization, titratable acidity was adjusted as necessary to 6.5 g/L TAE to create a microbially stable environment with the addition of tartaric acid.

3.2.3 Sampling Protocol

Samples were taken of post-crush must, pre-tannin addition, post-tannin addition, post alcoholic fermentation (PAF), at blend, post-blend, and post cold stabilization (CS). Post-crush must samples and any pre- or post- tannin addition samples involving solids contact were collected and strained through cheesecloth to remove solids. Soluble solids, titratable acidity, and pH were determined immediately for post-crush must samples. All samples were frozen at -20°C until analysis.

3.2.4 Analysis of Phenolics

Chemicals and Instrumentation

All solvents used, including methanol, acetonitrile, ethyl acetate, and formic acid, were HPLC grade (**Thermo-Fisher Scientific**, Waltham, MA). An Agilent Model 1260 Infinity series High Performance Liquid Chromatography (HPLC) (Palo Alto, CA) system with an in-line vacuum degasser, autosampler, binary pump, diode array detector, and thermostatted column compartment was used. The analysis required the use of two different HPLC columns: a Kinetex C18 column (100mm, 2.6µm particle size, 4.6mm inside diameter) and a Kinetex PFP (pentafluorophenyl) column (100mm, 2.6µm particle size, 2.1mm inside diameter), each fitted with a KrudKatcher guard filter (Phenomenex, Torrance, CA). Chromatographic analysis was

performed at a neighboring computer with Chemstation software (Version 3.04.02SP1 with spectral pack).

Sample Preparation

Must and wine samples were thawed and centrifuged for five minutes at 10,000 x g before undergoing direct injection and after dilution (for anthocyanins) or solid phase extraction (SPE). SPE followed a procedure developed by Jeffery et al. 2008 and modified by Manns and Mansfield (2012) and resulted in three fractions: monomeric compounds, anthocyanins, and polymeric tannins. Following SPE, or following a protocol of direct injection after dilution, monomeric and anthocyanin fractions were analyzed via HPLC using the method by Manns and Mansfield (2012). Polymeric tannin fractions were identified by following a phloroglucinolysis method (Manns and Mansfield 2012) as modified from Kennedy and Jones (2001).

Reversed-Phase HPLC of Phenolics

Monomeric compound, anthocyanin, and polymeric tannin fractions were analyzed using the Kinetex C18 column. Eluting flavan-3-ol monomers and polymeric substituents were identified and quantified using catechin, epicatechin, gallic acid, protocatechuic acid, dihydroxybenzoic acid, caffeic acid, coumaric acid, ferulic acid, ellagic acid, rutin, naringenin, and quercitin standards, directly from commercially available standards or semi-quantitatively. Anthocyanin identification and quantification was performed with the PFP column using malvidin-3-glucoside and malvidin-3,5-diglucoside standards as measures of equivalents for mono- and di-glucosides, respectively.

3.2.5 Statistical Analyses

Statistical data analysis was performed using analysis of variance (ANOVA) and TukeyHSD to find statistically different values between treatments at a significance level of <0.05 using R statistical software (Version 2.15.1).

3.3 Results and Discussion

3.3.1 Must and Wine Parameters

As cultivar treatments all originated from the same must, variability between duplicates and among treatments was low and not significant. Differences in post-fermentation and cold stabilization phenolic concentrations can, thus, be determined treatment effect. Must and wine parameters can be found in Table 3.3.1 and Table 3.3.2, respectively, and represent the duplicate fermentation averages.

Table 3.3.1. Must parameters for all cultivars. Data presented as an average of duplicates \pm standard deviation.

Grape Variety	Harvest Date	°Brix	pH	Titrateable Acidity (g/L)	YAN (mg N/L)
Maréchal Foch 1	09.05.2012	22.8	3.32	9.90	232
Maréchal Foch 2	09.05.2012	25.4	3.57	7.26	226
Arandell	09.17.2012	21.5	3.32	9.6	162
Corot noir	10.11.2012	19.6	3.31	7.89	130

Table 3.3.2. Wine composition after alcoholic fermentation of all cultivars and treatments. Data presented as an average of duplicates \pm standard deviation.

Maréchal Foch 1	pH	Titrateable Acidity (g/L)
BIO PRE-AF	3.69	6.54
BIO PRE-MLF	3.81	6.40
BIO POST-MLF	3.76	6.40
Maréchal Foch 2	pH	Titrateable Acidity (g/L)
BIO PRE-AF	4.06	5.24
BIO MID-AF	4.04	5.24
BIO POST-AF	4.03	5.37
BIO POST-MLF	4.06	5.41
NO BIO MLF	4.06	5.46
Arandell	pH	Titrateable Acidity (g/L)
BIO PRE-AF	3.89	5.56
BLEND	3.81	5.32
Corot noir	pH	Titrateable Acidity (g/L)
BIO PRE-AF	3.91	5.55
BIO MID-AF	3.91	5.75
BIO POST-AF	3.90	5.65
BLEND	3.82	5.15

3.3.2 Observed Phenolic Compounds

The concentrations of several phenolic compounds were followed throughout alcoholic and malolactic fermentation and cold stabilization (Table 3.3.3). These compounds were identified based on their detectability at certain wavelengths on two different columns (C18 and PFP) using HPLC. All results are presented as averages of the duplicate fermentations.

Table 3.3.3. Observed phenolic compounds in red hybrid winegrapes, viewed at optimal HPLC wavelengths.

280nm (C18)	320nm (C18)	360nm (C18)	520nm (PFP)
Gallic acid	Caffeic acid	Rutin	Cyanidin-3-glucoside
Protocatechuic acid	Caftaric acid	Myricetin	Cyanidin-3,5-diglucoside
Catechin	Coumaric acid	Quercetin	Delphinidin-3-glucoside
Epicatechin	Coutaric acid		Delphinidin-3,5-diglucoside
Ellagic Acid	Ferulic acid		Malvidin-3-glucoside
	Fertaric acid		Malvidin-3,5-diglucoside
	Sinapic acid		Peonidin-3-glucoside
	Caffeic acid ethyl ester		Peonidin-3,5-diglucoside
	Coumaric acid ethyl ester		Petunidin-3-glucoside
	Grape Reaction Product (GRP)		Petunidin-3,5-diglucoside

3.3.3 *Biotan Analysis*

Biotan was analyzed by HPLC for monomeric concentration and total tannin concentration at 1000 mg/L with phloroglucinolysis treatment. Monomeric analysis shows 10.48 ± 0.67 mg/L monomeric compounds (mostly catechin and epicatechin, but also containing procatechuic acid, gallic acid, and rutin). Average total tannin concentration of two samples was 234.09 ± 2.60 mg/L catechin equivalents with mDP values of 6.93 ± 0.04 , suggesting that condensed tannin makes up approximately 23.4% of Biotan. These results are much less than the total concentration found by Harbertson et al. (2012) to be 425 ± 1.65 mg/L catechin equivalents from a 1000 mg/L starting concentration, or 42.5%. The difference may arise from differences in analytical measurement, as Harberston et al. (2012) used a protein precipitation method, or may be due to batch-to-batch commercial tannin variation. HPLC analysis showed no epigallocatechin in Biotan, suggesting that condensed tannins from skins were not used for production, as epigallocatechin is a result of prodelphinidins that are only found in the skins.

Interestingly, the Biotan chromatogram also showed the presence of two atypical compounds, along with a less than expected concentration of epicatechin gallate (both in free form and phloroglucinol modified). Based on the retention times and position on the chromatogram, these peaks are expected to be catechin gallate-phloroglucinol and catechin gallate. Research on tea has shown that (-)-catechin gallate can be epimerized from (-)-epicatechin, (-)-epigallocatechin, (-)-epicatechin gallate, and (-)-epigallocatechin gallate when exposed to high temperatures (Seto et al. 1997; Ikeda et al. 2003). As exogenous tannins are steam or water extracted (Versari et al. 2012), a mechanism of high temperatures epimerization of grape condensed tannin would explain the presence of these compounds. One study found that gallocatechin gallate, which makes up less than 1.5 percent of catechins in brewed green tea,

comprised almost 50 percent of green tea extract in commercial beverages, likely as an epimerization product due to heat treatment (Chen et al. 2001). It is important to note that these peaks are not found in the 24 hour post-tannin addition chromatograms or any other post-tannin addition samples, suggesting that this monomer does not contribute to total tannin concentration and may, instead, be absorbed into cell wall fractions after addition.

3.3.4 Monomeric Phenolics

Maréchal Foch 1

Maréchal Foch 1 must contained less than 20mg/L of total monomerics (Table 3.3.4). After fermentation, all treatments reached concentrations just over 50mg/L, and monomeric concentration continued to rise in cold stabilized wines to concentrations between 145 to 160mg/L. Due to a shortage of fruit, however, it is difficult to draw conclusions from these results, as BIO PRE-MLF and BIO POST-MLF were produced in single lots, and there was no significant difference between cold stabilized wines. One interesting note, however, is that BIO POST-MLF increased only slightly in total monomeric concentration post-tannin addition when compared to BIO PRE-AF and BIO PRE-MLF, both of which nearly tripled in concentration after tannin addition. In these cases, catechin and caftaric acid concentrations played the most important role in the increased monomeric phenolics content. When tannin was added after MLF, there was little to no catechin and caftaric acid increase, possibly because the carboy was immediately moved to cooler temperature for cold stabilization. At cold temperatures, it is likely that the Biotan addition simply precipitated out of solution.

Table 3.3.4. Monomeric phenolics (mg/L) in Maréchal Foch1 must and wines as measured by HPLC. Data presented as an average of duplicates \pm standard deviation.

Stage	BIO PRE-AF	BIO PRE-MLF	BIO POST-MLF
<i>Must</i>	17.82 \pm 0.28	17.82 \pm 0.28	17.82 \pm 0.28
<i>Post-Tannin Addition (24hr)</i>	63.83 \pm 3.47	-	-
<i>PAF</i>	54.65 \pm 21.23	53.04	53.04
<i>Post-MLF</i>	-	-	148.95
<i>Post-Tannin Addition (24hr)</i>	-	142.15	153.27
<i>CS</i>	151.88 \pm 10.28	146.80	160.01

Post-MLF sample was taken only for BIO POST-MLF to record the monomeric concentration before tannin addition.

Maréchal Foch 2

Maréchal Foch 2 musts also had monomeric concentrations under 20 mg/L (Table 3.3.5). However, unlike the first lot, all Maréchal Foch 2 treatments reached over 70mg/L after fermentation except for the NO BIO treatment, which only reached 45mg/L. As treatments were split into 21 kg lots based on weight, this treatment may not have had as many solids as the first three treatments, which would decrease the amount of extractable monomeric compounds available. It is interesting to note that after tannin addition, all treatments except BIO POST-MLF dramatically increased in monomeric concentration. As in Maréchal Foch 1, however, after tannin addition to BIO POST-MLF these carboys were immediately put into cold stabilization, and the Biotan addition was potentially precipitated out of solution due to temperature change. Furthermore, the concentration remains relatively stable until cold stabilization, suggesting that there are few changes to monomeric concentration at this temperature.

BIO MID-AF had the highest levels of monomerics after fermentation, perhaps because exogenous tannin was added during fermentation and hydrolyzed. Catechin and gallic acid concentrations made up the majority of monomeric totals after fermentation for all treatments. After cold stabilization, BIO MID-AF had the highest concentration of monomerics, while BIO

PRE-AF had the lowest concentration of monomerics. Catechin, epicatechin, gallic acid, and caftaric acid were the dominating monomeric phenolics in wines of all treatments after cold stabilization. At cold stabilization, the BIO PRE-AF treatment had a lower monomeric concentration than both BIO MID-AF and BIO POST-MLF ($p < 0.01$). These differences come mainly from the variability in catechin, which is $90.76 \pm 4.51 \text{ mg/L}$, $114.79 \pm 8.77 \text{ mg/L}$, and $112.04 \pm 0.83 \text{ mg/L}$, respectively. These results could indicate that monomeric flavan-3-ols in BIO PRE-AF were involved in polymeric color formation and, thus, not free and quantifiable for HPLC analysis. This suggests that tannin addition to the must, as is a common practice, may be an effective way to increase color stability in wines (Boulton 2001).

Table 3.3.5. Monomeric phenolics (mg/L) in Maréchal Foch2 must and wines as measured by HPLC. Data presented as an average of duplicates \pm standard deviation.

Stage	BIO PRE-AF	BIO MID-AF	BIO POST-AF	BIO POST-MLF	NO BIO MLF
Must	18.79 \pm 1.79	16.43 \pm 0.11	16.33 \pm 0.91	16.88 \pm 0.27	16.88 \pm 0.27
Post-Tannin Addition	42.35 \pm 0.84*	-	-	-	-
Mid-AF	-	22.69 \pm 8.07	-	-	-
PAF	80.91 \pm 3.65	87.37 \pm 6.02	79.22 \pm 6.23	68.25 \pm 8.92	68.25 \pm 8.92
Post-MLF	-	-	-	230.84 \pm 3.49	-
Post-Tannin Addition	-	90.23 \pm 25.72*	202.36 \pm 7.52**	232.12 \pm 0.94*	-
CS	212.57 \pm 9.53 ⁺⁺	249.19 \pm 12.83 ⁺	222.67 \pm 0.23	246.79 \pm 2.48 ⁺	225.92 \pm 0.59

Mid-AF and Post-MLF samples were taken to assess the monomeric concentration before tannin addition for BIO MID-AF and BIO POST-MLF treatments, respectively.

⁺ Treatment-to-Treatment Significance at 0.01

* Sample taken 24 Hour Post Tannin-Addition

** Sample taken 72 Hour Post Tannin-Addition

Arandell

As Arandell is a relatively new cultivar, the table below (Tables 3.3.6), show specific compounds and their concentrations in must, post-alcoholic fermentation wine, and cold stabilized wine. Cold stabilized BIO PRE-AF wine was significantly higher in monomeric

compounds than the BLEND wine, as $p < 0.001$. This is expected, as the BLEND treatment has a 1:1 ratio of wine fermented without solid contact and wine fermented on solids. It is not surprising that the BLEND is not deficient in phenolic acids, as nonflavonoids make up the majority of phenolic acids in grape juice (Margalit 2004). The BIO PRE-AF treatment does, however, have notably higher amounts of catechin, the building block monomers of condensed tannins. BIO PRE-AF also has higher, though still low in concentration, amounts of the flavonol quercetin, a known copigmentation cofactor (Boulton 2001).

Table 3.3.6. Monomeric phenolics in Arandell must and wines as measured by HPLC. Data presented as an average of duplicates \pm standard deviation.

Compound, mg/L	MUST	PAF		CS	
	All Treatments	BIO PRE-AF	BLEND	BIO PRE-AF	BLEND
<i>Caffeic acid</i>	0.78 \pm 0.02	5.08 \pm 0.43	4.84 \pm 0.33	8.20 \pm 0.16	6.09 \pm 0.11
<i>Caffeic acid ethyl ester</i>	ND	0.34 \pm 0.10	0.29 \pm 0.01	1.37 \pm 0.05	0.78 \pm 0.02
<i>Caftaric acid</i>	0.02 \pm 0.01	20.81 \pm 1.08	17.24 \pm 1.44	23.63 \pm 1.25	17.23 \pm 0.25
<i>Catechin</i>	1.70 \pm 0.18	19.32 \pm 14.70	10.14 \pm 1.00	39.97 \pm 0.68	26.60 \pm 0.24
<i>Coumaric acid</i>	ND	1.25 \pm 0.09	1.14 \pm 0.09	4.23 \pm 0.10	2.93 \pm 0.08
<i>Coumaric acid ethyl ester</i>	ND	0.25 \pm 0.03	0.22 \pm 0.01	1.12 \pm 0.01	0.74 \pm 0.04
<i>t-Coutaric acid</i>	ND	6.05 \pm 1.30	3.77 \pm 0.03	8.58 \pm 0.20	5.40 \pm 0.41
<i>Ellagic acid</i>	0.64 \pm 0.10	1.13 \pm 0.33	1.03 \pm 0.16	0.88 \pm 0.13	0.72 \pm 0.17
<i>Epicatechin</i>	0.39 \pm 0.04	11.02 \pm 12.36	3.38 \pm 0.42	17.96 \pm 0.15	12.35 \pm 0.23
<i>t-Fertaric acid</i>	0.81 \pm 0.01	0.88 \pm 0.01	1.04 \pm 0.10	0.23 \pm 0.06	0.08 \pm 0.01
<i>Ferulic acid</i>	0.01 \pm 0.00	0.22 \pm 0.04	0.22 \pm 0.03	0.87 \pm 0.04	0.52 \pm 0.07
<i>Gallic acid</i>	ND	5.66 \pm 1.15	5.46 \pm 0.42	7.19 \pm 0.10	5.42 \pm 0.11
<i>GRP</i>	3.91 \pm 0.11	2.27 \pm 0.54	5.22 \pm 0.44	1.28 \pm 0.16	2.77 \pm 0.00
<i>Protocatechuic acid</i>	0.20 \pm 0.13	2.35 \pm 0.94	2.59 \pm 0.02	1.83 \pm 0.02	1.36 \pm 0.04
<i>Quercetin</i>	ND	0.63 \pm 0.32	0.24 \pm 0.25	5.08 \pm 1.01	1.43 \pm 0.08
<i>Quercetin-3-Glucuronide</i>	ND	4.64 \pm 0.57	2.74 \pm 0.31	3.75 \pm 0.46	1.39 \pm 0.09
<i>Quercetin-3-Glucoside</i>	ND	0.64 \pm 0.06	0.45 \pm 0.13	0.90 \pm 0.08	0.32 \pm 0.09
<i>Quercetin-3-Rhamnoside</i>	ND	0.92 \pm 1.30	ND	ND	ND
<i>Myricetin</i>	ND	0.39 \pm 0.07	0.19 \pm 0.01	ND	ND
<i>Sinapic acid</i>	ND	0.11 \pm 0.01	0.14 \pm 0.04	0.17 \pm 0.02	0.07 \pm 0.00
<i>Rutin</i>	0.51 \pm 0.01	1.62 \pm 0.35	0.99 \pm 0.12	2.04 \pm 0.45	0.83 \pm 0.02
Total Monomerics	8.97\pm0.03	85.59\pm28.53	61.33\pm3.60	129.29\pm2.51⁺	87.03\pm0.39⁺

⁺Treatment-to-Treatment Significance at 0.001

ND = not detectable

Corot noir

Corot noir shows very low concentrations of monomeric compounds in musts, with approximately 5-7mg/L total monomerics (Table 3.3.7). Grape reaction product (GRP) makes up the majority of this fraction, accounting for over 50% of total monomeric concentration for each treatment. GRP can occur from a reaction involving caftaric acid or coutaric acid in must when oxygen is present (Singleton et al. 1985). After fermentation, total monomeric concentrations

ranged from about 37-81mg/L. After cold stabilization, the concentration of total monomerics increased for each treatment. The BLEND is significantly lower in monomeric compounds than all other treatments after cold stabilization at $p < 0.001$. It is interesting to note that the control (BIO PRE-AF) had a significantly higher concentration of total monomerics after fermentation, but is not significantly different from BIO MID-AF or BIO POST-AF after cold stabilization. The control also showed the smallest increase in total monomerics concentration between fermentation and cold stabilization. The blend, with the lowest concentration of total monomerics at cold stabilization, has the highest percentage of increase in concentration from fermentation to cold stabilization, comprised mostly of condensed tannin building blocks catechin and epicatechin, and caffeic acid, a possible copigmentation cofactor (Boulton 2001). While the monomeric compounds that make up BIO PRE-AF, BIO MID-AF, and BIO POST-AF mostly consist of catechin, epicatechin, and coumaric acid, these treatments also have much higher amounts of caftaric acid in final wines than the BLEND (20mg/L and 0.20mg/L, respectively). This increase in caftaric acid could mean that there was little oxidation throughout the fermentation, as high amounts of oxidation result in major loss of caftaric acid (Singleton et al. 1985), but, as the monomeric concentration of the must was largely GRP, a product of caftaric acid in the presence of oxygen, this explanation is unlikely. It could also be possible that exogenous tannin contained high amounts of caftaric acid, as it is present not only in grape pulp, but also in seeds and skins (Margalit 2004). However, after Biotan analysis showed non-detectable concentrations of caftaric acid, this explanation is also unlikely. Perhaps a better explanation is that caftaric acid acted as a copigment with anthocyanins, (Boulton 2001) and, as the BLEND was half composed of wine with no skin contact, there were fewer copigmentation reactions. A second explanation could be that major monomeric compounds found in the

BLEND were caffeic acid and coumaric acid, as well as catechin, and epicatechin, suggesting that the cinnamic acids in the blend are more commonly found non-esterified.

Table 3.3.7. Monomeric phenolics (mg/L) in Corot noir musts and wines as measured by HPLC. Data presented as an average of duplicates \pm standard deviation.

Stage	BIO PRE-AF	BIO MID-AF	BIO POST-AF	BLEND RASW/NO BIO
<i>Must</i>	7.24 \pm 0.45	6.66 \pm 0.01	6.54 \pm 0.53	5.93 \pm 1.04/6.96 \pm 0.19
<i>Post-Tannin Addition (24hr)</i>	28.43 \pm 2.24	-	-	-
<i>Mid-AF</i>	-	54.02 \pm 4.30	-	-/-
<i>Post-Tannin Addition (24hr)</i>	-	63.19 \pm 9.55	79.61 \pm 13.04	15.08 \pm 2.29/-
<i>PAF</i>	81.55 \pm 0.21	68.94 \pm 4.07	79.61 \pm 13.04	36.94 \pm 1.06
<i>CS</i>	97.87 \pm 1.75 ⁺	96.68 \pm 3.31 ⁺	91.84 \pm 7.32 ⁺	53.79 \pm 2.25 ⁺⁺⁺

MID-AF sample was taken only for BIO POST-MLF to record the monomeric concentration before tannin addition.

⁺ Treatment-to-Treatment Significance at 0.001

Cultivar Comparison

While there was no significant difference between monomeric concentrations in Maréchal Foch 1 wines after cold stabilization, there was evidence, seen in both Maréchal Foch 1 and Maréchal Foch 2, that cold temperatures may cause early loss of exogenous tannin. In Arandell and Corot noir treatments, the BLEND cold stabilized wine was significantly lower than other treatments, which may be a result of lower tannin concentrations in the BLEND than other treatments (200 mg/L versus 400 mg/L, respectively). Caftaric acid amounts were similar to the control in the Arandell BLEND, while Corot noir saw very reduced levels of caftaric acid in comparison to other treatments. This may be a result of the amount of caftaric acid present in the berry or winemaking practices, including oxygen exposure (Margalit 2004; Singleton et al. 1985). In all cultivars, BIO PRE-AF treatments had similar or lower monomeric concentrations (composed primarily of catechin and epicatechin) than other treatments that followed maceration and tannin addition (with the exception of Arandell as there is no comparable treatment). This

suggests that tannin addition to must may result in more monomeric flavan-3-ols being polymerized in cold stabilized wines rather than existing as free monomers (Boulton 2001). This could be important to producers, as it is often the manufacturer's suggestion to add tannin before fermentation, and polymeric color formation is associated with color stability in wines.

3.3.5 Anthocyanins

Maréchal Foch 1

Anthocyanin concentration in Maréchal Foch 1 increased after fermentation (Table 3.3.8). Concentrations of anthocyanins after fermentation were not significantly different across the control, BIO PRE-MLF, and BIO POST-MLF. Although BIO PRE-MLF and BIO POST-MLF were done in singlet and can only be used to draw limited conclusions, it is interesting to note the low ratio of monoglucosides to diglucosides in these treatments, compared to the higher concentration of monoglucosides in the BIO PRE-AF treatment. These concentrations mirror that found in Manns et al. (2013), and may be attributed to the strong *V. vinifera* genetic heritage.

Table 3.3.8. Anthocyanins (mg/L) in Maréchal Foch 1 must and wines as measured by HPLC. Data presented as an average of duplicates \pm standard deviation.

Stage	BIO PRE-AF (Mono/Di)	BIO PRE- MLF (Mono/Di)	BIO POST- MLF (Mono/Di)
<i>Must</i>	108.67 \pm 1.31	108.67 \pm 1.31	108.67 \pm 1.31
<i>Post-Tannin Addition (24 hr)</i>	260.81 \pm 49.88	-	-
<i>PAF</i>	82.40 \pm 8.60	84.77	84.77
<i>Post-MLF</i>	-	-	183.27
<i>Post-Tannin Addition (24 hr)</i>	-	258.05	164.41
<i>CS</i>	176.62 \pm 13.93 (70.80 \pm 6.66/66.82 \pm 3.80)	154.09 (31.50/78.83)	152.27 (30.35/78.10)

POST-MLF sample was taken only for BIO POST-MLF to record the monomeric concentration before tannin addition.

Mono: monoglucoside

Di: diglucoside

Maréchal Foch 2

Anthocyanin concentrations in Maréchal Foch increased between fermentation and cold stabilization for all treatments (Table 3.3.9). All final anthocyanin concentrations were around 300mg/L, suggesting that tannin addition timing may not effect final anthocyanin concentration, as NO BIO MLF had similar anthocyanin levels as treatments with tannin addition. Skin contact is crucial to increase anthocyanin concentration, and all treatments had identical maceration time. While there were no significant differences among treatments, it is important to note that, again, the monoglucoside concentration is nearly equal to the diglucoside concentration for all treatments, as was observed by Manns et al. (2013).

Table 3.3.9. Anthocyanins in Maréchal Foch2 must and wines as measured by HPLC. Data presented as an average of duplicates \pm standard deviation.

Stage	BIO PRE-AF (Mono/Di)	BIO MID-AF (Mono/Di)	BIO POST-AF (Mono/Di)	BIO POST-MLF (Mono/Di)	NO BIO MLF (Mono/Di)
Must	134.34 \pm 1.02	130.09 \pm 4.36	129.08 \pm 0.56	141.27 \pm 3.87	141.27 \pm 3.87
Post-Tannin Addition	331.65 \pm 21.01*	-	-	-	-
Mid-AF	-	267.29 \pm 10.85	-	-	-
Post-MLF	-	-	-	331.28 \pm 1.77	-
Post-Tannin Addition	-	408.69 \pm 7.26*	387.66 \pm 22.53**	330.70 \pm 0.87*	-
PAF	153.37 \pm 9.73	152.87 \pm 6.53	147.15 \pm 3.69	141.62 \pm 7.94	141.62 \pm 7.94
	301.48 \pm 6.06	304.47 \pm 7.92	298.07 \pm 5.37	307.53 \pm 14.10	307.49 \pm 1.53
CS	(120.47 \pm 3.46/ 117.41 \pm 1.05)	(120.27 \pm 0.66/ 119.35 \pm 5.52)	(117.97 \pm 3.07/ 116.23 \pm 1.03)	(123.77 \pm 5.43/ 122.22 \pm 5.81)	(125.28 \pm 0.11/ 122.30 \pm 0.73)

Mid-AF and Post-MLF samples were taken to assess the monomeric concentration before tannin addition for BIO MID-AF and BIO POST-MLF treatments, respectively.

** Sample taken 24 Hour Post Tannin-Addition*

*** Sample taken 72 Hour Post Tannin-Addition*

Mono: monoglucoside

Di: diglucoside

Arandell

Concentrations of anthocyanins in Arandell must are very low, but increase substantially in cold stabilized wines (Tables 3.3.10). There is a significantly higher anthocyanin concentration in the control than BLEND at $p < 0.01$. This is probably attributed to the fact that half of the blend originates from a wine that fermented without skin contact. Diglucosides are the dominant form of anthocyanins in both treatments.

Table 3.3.10. Anthocyanins in Arandell must as measured by HPLC. Data presented as an average of duplicates \pm standard deviation.

Compound, mg/L	MUST	PAF		CS	
	All Treatments	BIO PRE-AF	BLEND	BIO PRE-AF (Mono/Di)	BLEND (Mono/Di)
<i>Delphinidin-3,5-DiGlucoside</i>	ND	6.93 \pm 3.13	3.38 \pm 0.17	8.72 \pm 0.08	4.85 \pm 0.03
<i>Cyanidin-3,5-DiGlucoside</i>	ND	4.78 \pm 1.46	2.67 \pm 0.18	5.00 \pm 0.29	3.06 \pm 0.15
<i>Petunidin-3,5-DiGlucoside</i>	ND	11.85 \pm 4.262	5.31 \pm 0.02	13.45 \pm 0.09	4.57 \pm 6.46
<i>Delphinidin-3-Glucoside</i>	ND	9.06 \pm 7.45	2.90 \pm 0.10	14.27 \pm 0.38	12.20 \pm 1.50
<i>Peonidin-3,5-DiGlucoside</i>	7.21 \pm 0.01	61.25 \pm 20.27	35.75 \pm 2.25	64.10 \pm 0.99	42.84 \pm 2.87
<i>Cyanidin-3-Glucoside</i>	0.83 \pm 0.00	2.04 \pm 1.18	1.07 \pm 0.04	1.88 \pm 0.03	1.39 \pm 0.11
<i>Malvidin-3,5-DiGlucoside</i>	9.01 \pm 0.14	129.53 \pm 46.96	69.35 \pm 4.38	136.43 \pm 1.06	88.03 \pm 5.44
<i>Petunidin-3-Glucoside</i>	ND	11.22 \pm 9.92	3.70 \pm 0.14	18.79 \pm 0.43	14.15 \pm 1.41
<i>Peonidin-3-Glucoside</i>	3.61 \pm 0.07	8.88 \pm 7.72	3.34 \pm 0.04	11.24 \pm 0.15	8.47 \pm 0.81
<i>Malvidin-3-Glucoside</i>	5.76 \pm 0.13	57.56 \pm 53.66	20.05 \pm 0.88	93.88 \pm 1.07 20.40 \pm 0.09	70.44 \pm 6.49 12.57 \pm 0.70
Total Anthocyanins	26.42 \pm 0.33	15.72 \pm 5.15 318.82 \pm 161.16	8.71 \pm 0.76 156.24 \pm 8.96	388.17 \pm 0.25 ⁺ (140.07 \pm 1.99/ 227.71 \pm 2.33)	262.55 \pm 22.61 ⁺ (106.65 \pm 7.32/ 143.34 \pm 14.89)

⁺ Treatment-to-Treatment Significance at 0.01

Mono: monoglucoside

Di: diglucoside

Corot noir

Total anthocyanin concentration in Corot noir must was below 50 mg/L and dominated by peonidin-3,5-diglucoside and malvidin-3,5-diglucoside (Table 3.3.11). After fermentation, the

control treatment showed the highest concentration of phenolics, reaching over 600mg/L. BIO MID-AF, which was pressed from skins before completing fermentation, had the lowest anthocyanin concentration after fermentation, but reached concentrations similar to BIO PRE-AF and BIO POST-AF as a finished wine. This is expected as anthocyanin concentrations peak a few days into fermentation (Kennedy and Peyrot des Gachons 2003). After cold stabilization, all treatments increased in total anthocyanin concentration; the control increased the least ($>0.3\%$), while the blend increased the most by over 37%. After cold stabilization, there was significant difference of $p < 0.001$ between the BLEND and all other treatments, with the BLEND having a significantly lower anthocyanin concentration than all other treatments. It is also interesting to note the preference of the diglucoside form for all finished wines.

While the BLEND treatment had significantly lower amounts of anthocyanins than all other treatments, those three treatments did not have any significant differences among themselves. This confirms the importance of skin contact during maceration, which was the same in BIO PRE-AF, BIO MID-AF, and BIO POST-AF. It is important to note that the blend has a slightly higher monoglucosides to diglucosides ratio than the other three treatments.

Table 3.3.11. Anthocyanins in Corot noir must and wines as measured by HPLC. Data presented as an average of duplicates \pm standard deviation.

Stage	BIO PRE-AF (Mono/Di)	BIO MID-AF (Mono/Di)	BIO POST-AF (Mono/Di)	BLEND RASW/NO BIO (Mono/Di)
Must	41.20 \pm 0.76	41.22 \pm 0.36	40.39 \pm 0.40	47.06 \pm 5.54/41.93 \pm 1.76
Mid-AF	-	595.32 \pm 24.23	-	-
Post-Tannin Addition (24hr)	279.59 \pm 10.71	536.00 \pm 70.90	-	62.23 \pm 19.19/-
PAF	609.60 \pm 23.15	536.58 \pm 5.76	567.01 \pm 35.56	257.38 \pm 0.89
Post-Tannin Addition (24hr)	-	-	533.89 \pm 10.23	-
	611.62 \pm 10.76 ⁺	586.07 \pm 27.02 ⁺	583.98 \pm 11.58 ⁺	353.60 \pm 36.82 ⁺⁺⁺
CS	(48.22 \pm 2.31/ 478.79 \pm 7.71)	(50.27 \pm 3.65/ 454.07 \pm 20.43)	(44.94 \pm 3.63/ 462.20 \pm 4.01)	(34.71 \pm 3.27/ 270.14 \pm 26.99)

⁺ Treatment-to-Treatment Significance at $p < 0.001$

Mono: monoglucoside

Di: diglucoside

Cultivar Comparison

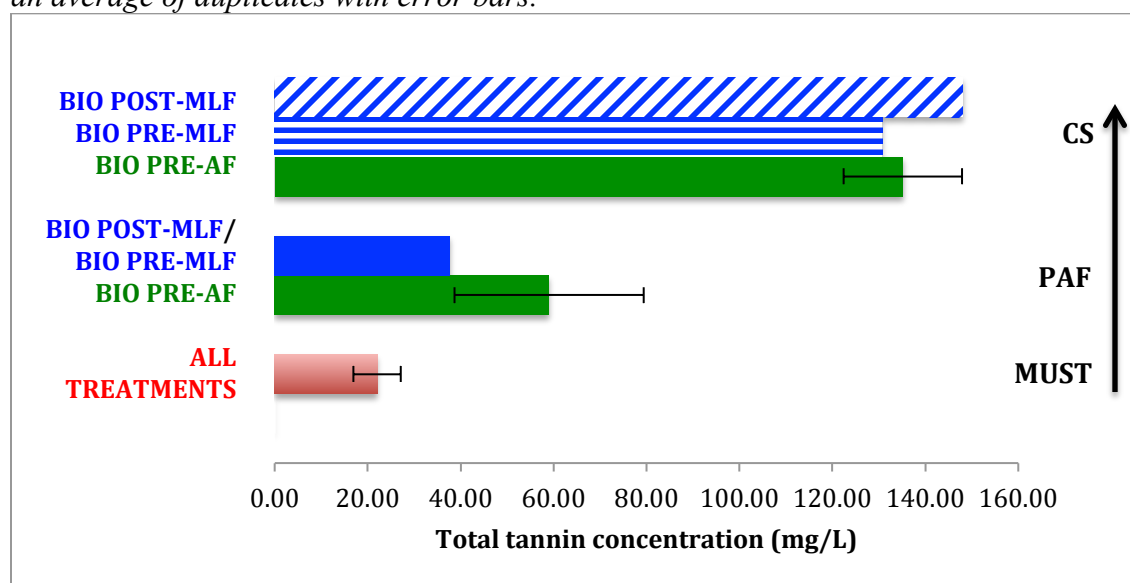
Anthocyanins in *V. vinifera* grapes range from 500 to 1,200 mg/kg based on cultivar, maturity, climate, location, and fruit yield (Kennedy and Peyrot des Gachons 2003) and the concentration in these wines varies based on the original grape concentration and extraction technique (Monagas and Bartolomé 2003). Maréchal Foch, in general, had higher anthocyanin concentrations in the must, and had an almost equal monoglucoside to diglucoside ratio, most likely a result of the strong *V. vinifera* genetic background. Arandell and Corot noir, however, had low must concentrations and very high amounts of the diglucoside form. This phenomenon of selective extraction is similar to observations made by Mann et al. (2013). BLEND treatments had significantly lower concentrations of anthocyanins, as expected with half of the wine from red grapes fermented without skin contact, but tannin treatment showed no differences in anthocyanin concentrations among treatments.

3.3.6 Condensed Tannin

Maréchal Foch 1

Tannin concentrations in Maréchal Foch wine were greater than 120mg/L for all treatments (Figure 3.3.1). BIO POST-MLF showed the highest concentration of total tannin at almost 150mg/L, BIO PRE-MLF and BIO PRE-AF have similar concentrations around 140mg/L. While there were no significant differences in total tannin concentration among treatments, there was an increase in tannin concentration for all treatments after cold stabilization, which may be a result of the exogenous tannin addition.

Figure 3.3.1. Total tannin concentration in Maréchal Foch 1 musts and wines. Data presented as an average of duplicates with error bars.

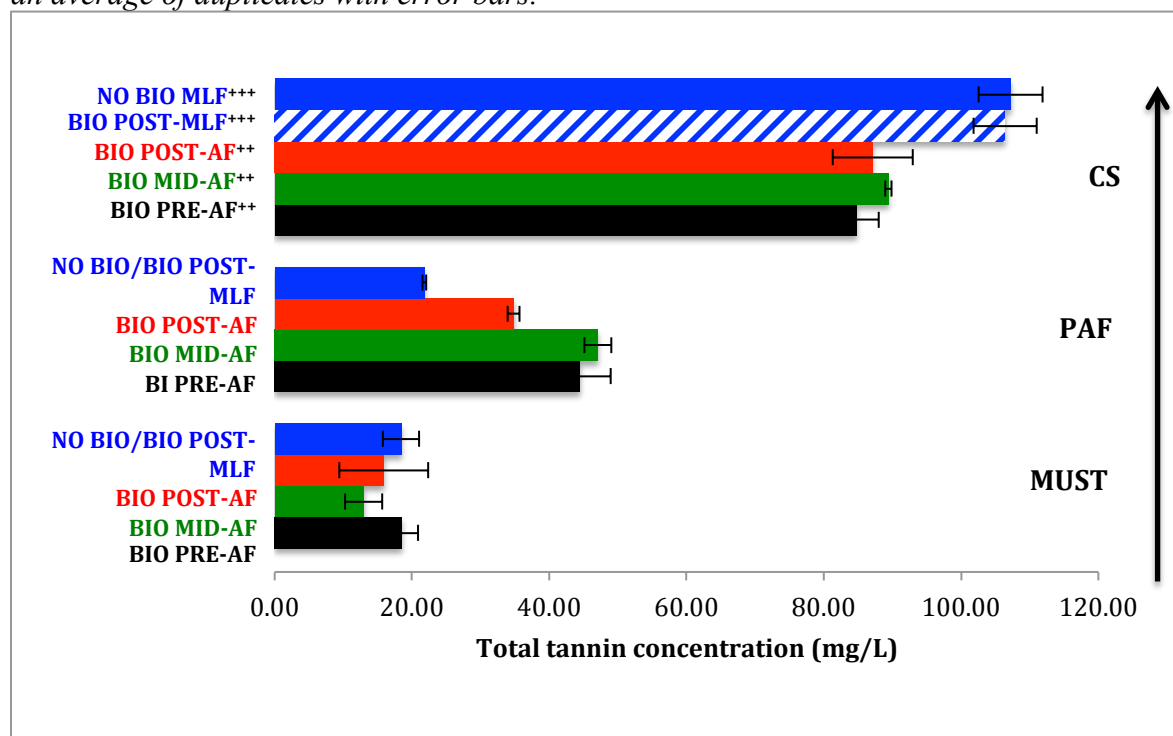


Maréchal Foch 2

The concentration of tannins was below 120 mg/L in all treatments (Figure 3.3.2) but did show an increase in concentration from musts to cold stabilized wines. NO BIO MLF and BIO POST-MLF had significantly higher tannin concentration in cold stabilized wines than all other treatments. This could suggest that later timing increases total tannin concentration; however, the NO BIO MLF treatment, which received no tannin addition, had significantly higher total tannin

concentrations. This could suggest that Biotan additions added around alcoholic fermentation are being used to form polymeric color compounds with anthocyanins, as Maréchal Foch 2 treatments that are lowest in tannin concentration are also lowest in anthocyanin concentration. In this case, these tannins involved in polymeric color would not be free and quantifiable in total tannin concentration.

Figure 3.3.2. Total tannin concentration in Maréchal Foch 2 musts and wines. Data presented as an average of duplicates with error bars.



⁺⁺ Treatment-to-Treatment Significance from NO BIO MLF AND BIO POST-MLF at $p < 0.05$

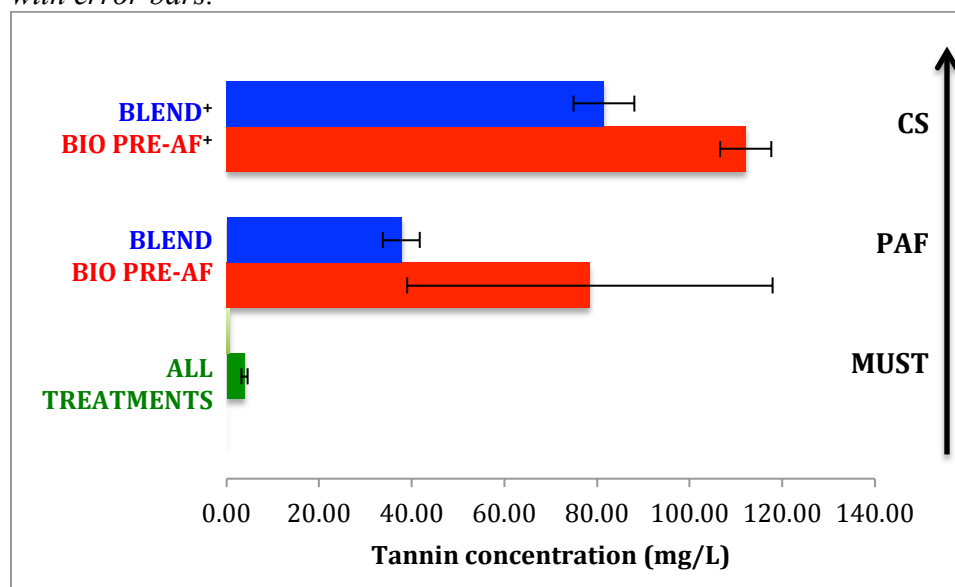
⁺⁺⁺ Treatment-to-Treatment Significance from BIO POST-AF, BIO MID-AF, AND BIO PRE-AF at $p < 0.05$

Arandell

Arandell must had extremely low tannin concentration in must and wines, but concentrations, while still low, nearly doubled from fermentation to cold stabilized wines. (Figure 3.3.3). The BLEND cold stabilized wine treatments had a significantly lower tannin concentration than BIO PRE-AF at $p < 0.01$. It is important to note that BLEND wines had 200

ppm Biotan concentration, as compared to 400 ppm in the control, and this may impact the low total tannin concentration in the cold stabilized wines.

Figure 3.3.3. Total tannin concentration in Arandell. Data presented as an average of duplicates with error bars.



⁺ Significance at 0.01

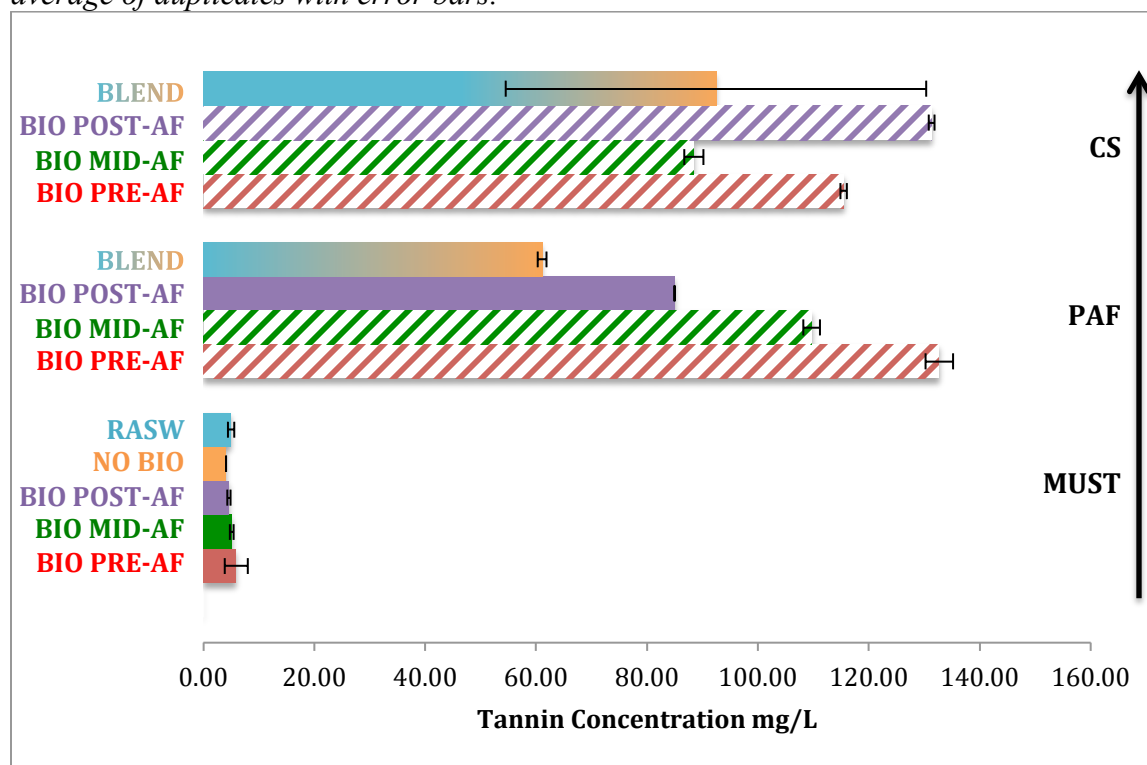
Corot noir

Tannin concentrations in Corot noir increased dramatically from must concentrations to cold stabilized wine (Figure 3.3.4). After fermentation, the control wine had the highest concentration of tannins, with over 120mg/L. As cold stabilized wines, BIO POST-AF had the greatest concentration of tannins, while BIO MID-AF had the lowest concentration, yet there were no significant differences among treatments. However, results suggest that adding tannin later in the winemaking process may provide the highest tannin concentration in the cold stabilized wine as both BIO PRE-AF and BIO MID-AF decreased in tannin concentration from fermentation to cold stabilized wines. This result may support the idea that the treatments lower in total tannin concentration have higher polymeric color concentration than other treatments.

The BLEND treatment had a very high standard deviation, making it hard to draw conclusions from the data. Looking deeper into analysis shows that one of the two BLEND

duplicates had almost twice the amount of catechin and epicatechin terminal units and nearly twice the amount of catechin, epicatechin, and epicatechin gallate extension units. As the BLEND treatment had 200 ppm Biotan concentration, it is likely that there was error during HPLC sample preparation or analysis and the blend had a significantly lower tannin concentration than other treatments, as is observed in the Arandell BLEND wines.

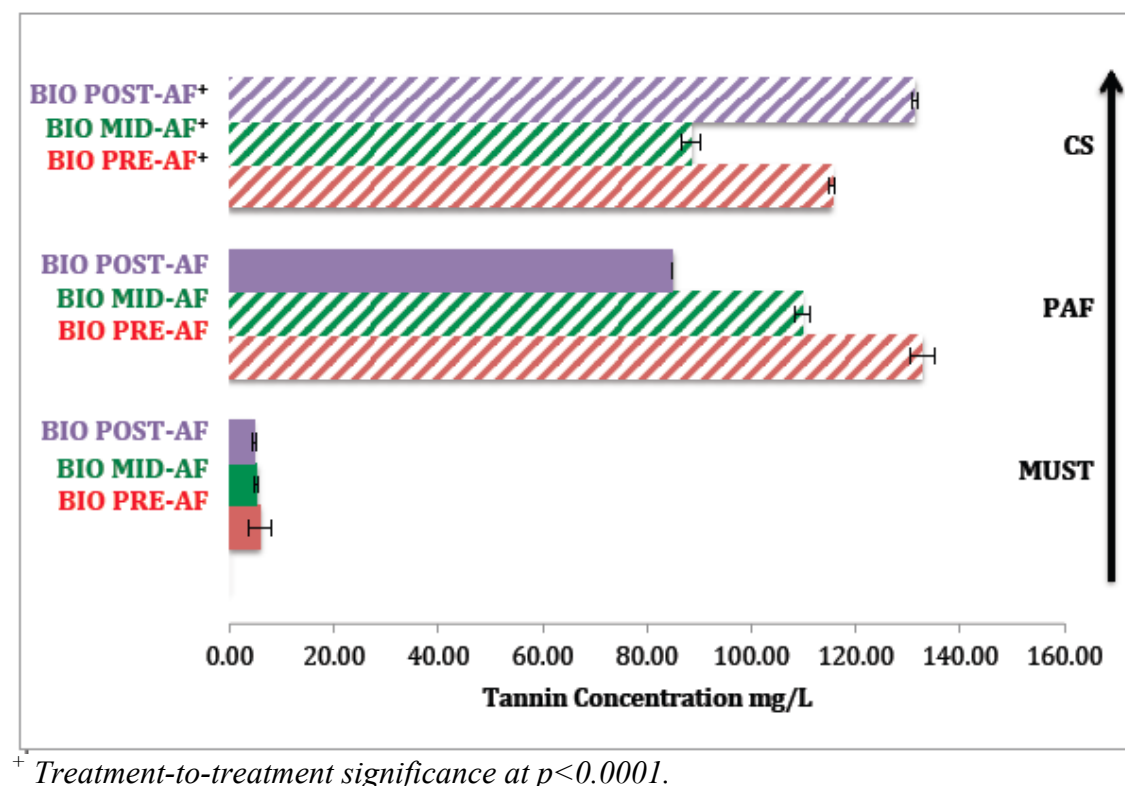
Figure 3.3.4. Total tannin concentration in Corot noir musts and wines. Data presented as an average of duplicates with error bars.



In order to better identify significance between the BIO POST-AF, BIO MID-AF, and BIO PRE-AF treatments, the BLEND treatment was removed (Figure 3.3.5). In this case, BIO POST-AF had a significantly higher total tannin concentration than BIO MID-AF and BIO PRE-AF. This suggests that later tannin addition can increase total tannin concentration. BIO PRE-AF also had a higher total tannin concentration than BIO MID-AF. This result can be explained by the fact that BIO MID-AF was pressed from solids before fermentation finished and probably was not able to extract as much seed tannin, as seed tannin concentration increases with

increased ethanol concentrations and, thus, increased fermentation time (Kennedy and Peyrot des Gachons 2003).

Figure 3.3.5. Total tannin concentration in Corot noir musts and wines. Data presented as an average of duplicates with error bars.



Cultivar Comparison

All wines had low total tannin concentrations when compared to *V. vinifera* wines, which average 544 mg/L (Harbertson et al. 2008). Similar total tannin concentrations were also found by Manns et al. (2013) when comparing exogenous tannin treated Maréchal Foch and Corot noir wines, with differences possibly attributed to vintage. In all cultivars, total tannin concentrations increased dramatically in cold stabilized samples, except BIO MID-AF and BIO PRE-AF Corot noir treatments. This may simply be a cause of different grape chemistry or more polymeric color formation in these treatments. The results may suggest that exogenous tannin addition later in the winemaking process could lead to a higher total tannin concentration, as BIO POST-MLF

in Maréchal Foch and BIO POST-AF in Corot noir show higher concentration in cold stabilized wines.

3.3.6 Polymeric Color

Polymeric color analysis showed that concentrations were highest in control wines (BT PRE-AF) in all cultivars, although there was no significant difference between control wines and treatments in any cultivar; nor was there significance among treatments. In cultivars such as Maréchal Foch 2, where BT PRE-AF treatments also saw low monomeric and total tannin concentration, this result could suggest that the Biotan addition is being used to form more polymeric color (Table 3.3.12).

Table 3.3.12. Polymeric color concentration of cold stabilized wine samples across cultivars. Data presented as an average of duplicates \pm standard deviation.

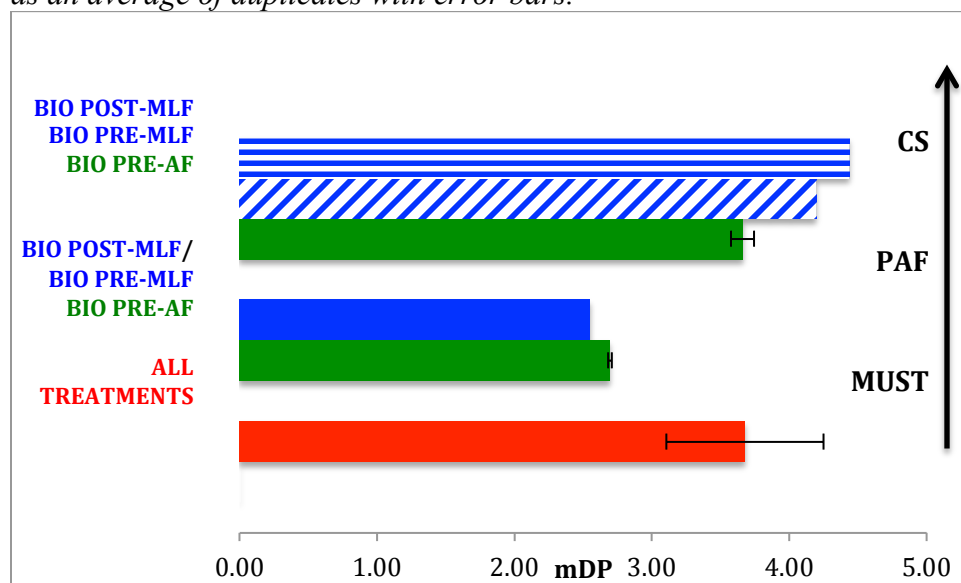
Cultivar	Treatment	Polymeric Color (mg/L)
Maréchal Foch 1	BT PRE-AF	20.95 \pm 1.36
	BT PRE-MLF	16.40
	BT POST-MLF	17.34
Maréchal Foch 2	BT PRE-AF	27.01 \pm 3.34
	BT MID-AF	24.93 \pm 0.17
	BT POST-AF	24.14 \pm 0.71
	BT POST-MLF	25.29 \pm 0.95
	NO BT	28.30 \pm 2.57
Arandell	BT PRE-AF	22.71 \pm 0.71
	BLEND	15.82 \pm 3.42
Corot noir	BT PRE-AF	27.84 \pm 2.64
	BT MID-AF	24.09 \pm 0.57
	BT POST-AF	25.26 \pm 2.01
	BLEND	23.21 \pm 10.32

3.3.7 Mean Degree of Polymerization (mDP)

Maréchal Foch 1

The mean degree of polymerization in Maréchal Foch 1 remained about the same in cold stabilized wines and musts (Figure 3.3.6). The control had the lowest mDP, but there was no significance among treatments.

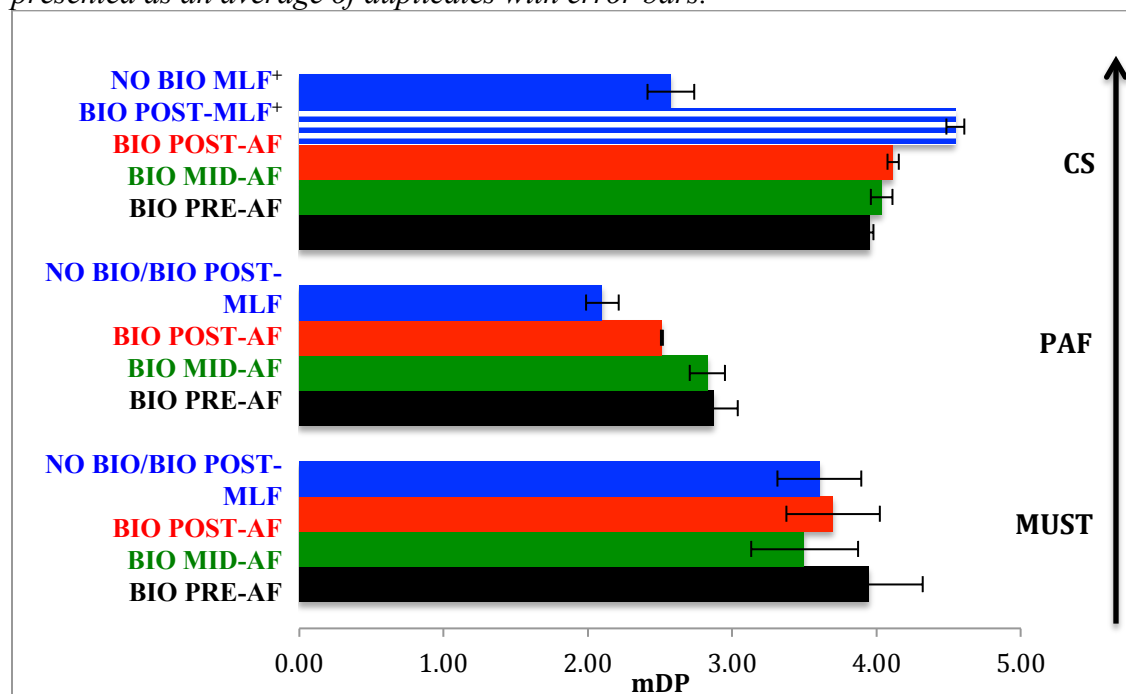
Figure 3.3.6. Mean degree of polymerization in Maréchal Foch musts and wines. Data presented as an average of duplicates with error bars.



Maréchal Foch 2

Maréchal Foch 2 shows mDP around 4 in cold stabilized wines (Figure 3.3.7) except in the NO BIO MLF treatment. NO BIO MLF had a significantly lower mDP than all treatments, while BIO POST-MLF had a significantly higher mDP than all treatments. These results may suggest not only that tannin addition helps to increase mDP of condensed tannins, but also that these mDP values may increase with later tannin addition. The wines need to be sampled after time to see if this mDP lasts in aged wines.

Figure 3.3.7. Mean degree of polymerization in Maréchal Foch 2 musts and wines. Data presented as an average of duplicates with error bars.

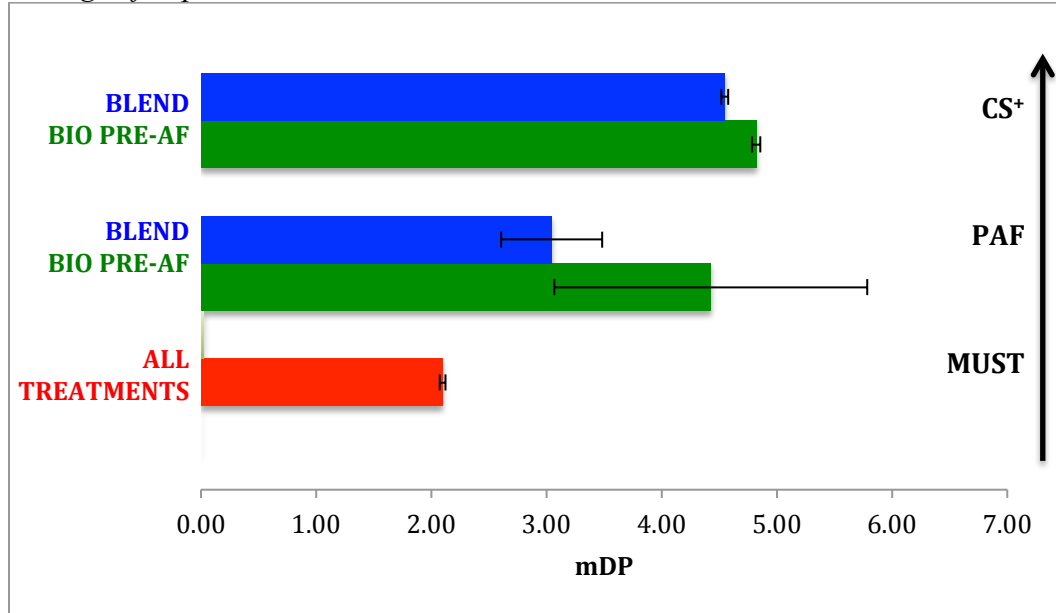


⁺ Treatment-to-treatment significance between NO BIO MLF and all other treatments. BIO POST-MLF and all other treatments, and NO BIO MLF AND BIO POST-MLF at $p < 0.0001$, < 0.01 (< 0.05 between BIO POST-MLF AND BIO POST-AF), and < 0.0001 , respectively

Arandell

Arandell mDP units were low in cold stabilized wines (Figure 3.3.8). BIO PRE-AF had a significantly higher mDP than the BLEND treatment after cold stabilization, but probably not enough to dramatically change sensory characteristics of bitterness to astringency.

Figure 3.3.8. Mean degree of polymerization in Arandell musts and wines. Data presented as an average of duplicates with error bars.

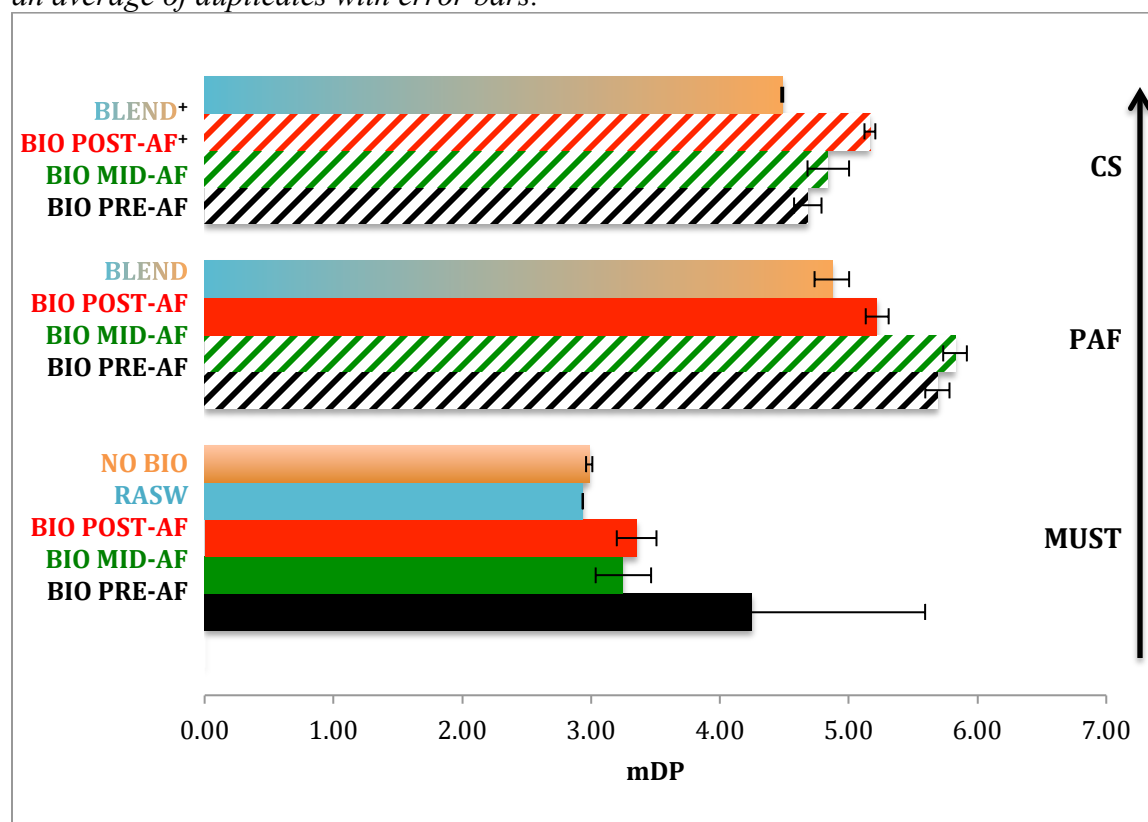


⁺ Treatment-to-treatment Significance at 0.01

Corot noir

Mean degree of polymerization was low in all must treatments (Figure 3.3.9). All mean degree of polymerization values decreased after fermentation. BIO POST-AF had a significantly higher mDP than the BLEND. This could suggest that later exogenous tannin addition increases mean degree of polymerization.

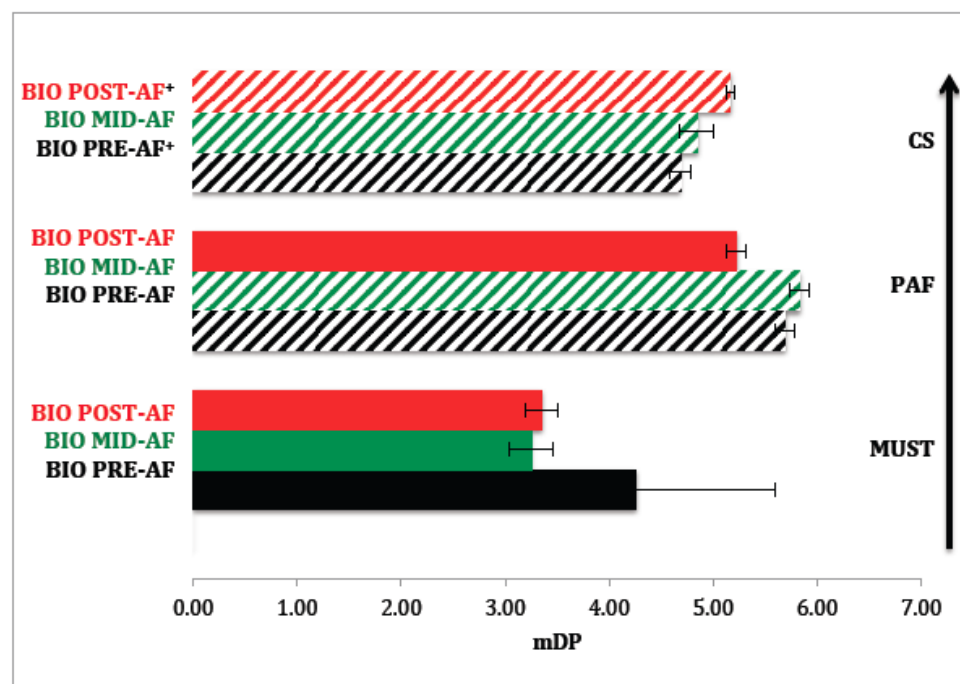
Figure 3.3.9. Mean degree of polymerization in Corot noir musts and wines. Data presented as an average of duplicates with error bars.



⁺ Treatment-to Treatment Significance at 0.001

In order to better compare significance between treatments BIO POST-AF, BIO MID-AF, and BIO PRE-AF, the BLEND treatment was removed from analysis (Figure 3.3.10). The results show that BIO POST-AF has a significantly higher mDP than BIO PRE-AF. This suggests that later tannin addition increases the mean degree of polymerization.

Figure 3.3.10. Mean degree of polymerization in Corot noir musts and wines. Data presented as an average of duplicates with error bars.



⁺ Treatment-to-treatment Significance at $p < 0.05$.

Cultivar Comparison

Musts and wines across cultivars had low mean degrees of polymerization values. The highest mDP reached almost six. One trend noticed between cultivars was that later tannin addition encouraged higher mDP values in all treatments (excluding Arandell). The BLEND consistently had the lowest mDP of all treatments, though not significantly lower than all treatments in Corot noir. Mean degree of polymerization is associated with sensory characteristics of the finished wine in that low mDPs contribute more bitterness, while high mDPs enhance astringency (Peleg et al. 1999). The mDP of these cultivars are all very low, suggesting a bitter wine to be expected. The mDP should be measured after these wines age to conclude whether mDP values remain higher in wines with a later Biotan addition.

3.4 Conclusion

Tannin addition research has expanded in the past few years as studies question the timing, amount, and overall impact of the practice. The changes reported in this study may not be significant enough to effect wine sensory properties. While tannin addition varies according to grape characteristics (Canuti et al 2012), hybrid winegrapes already lack tannins, and are generally expected to benefit from increased concentrations of condensed tannin. The effects seen in this study suggested possible benefits from later tannin addition; however, the wines studied were very young, and the effect of tannin additions on long-term color stability are unknown. As hybrids contain monoglucoside as well as diglucoside anthocyanin forms, color stability may be attributed to the copigmentation affects between diglucosides and other monomeric phenolics, as malvidin-3,5-diglucoside has greater color increase associated with copigmentation cofactors than malvidin-3-glucoside (Boulton 2001). However, quercetin, found as the most effective copigment, was present only in low concentrations, while catechin, a poor copigment, was evident in high concentration (Lambert et al. 2001). This may mean that copigmentation in these hybrid wines relies on self-association between diglucosides to maintain color stability. Copigmentation, however, decreased by 55% after 11 months in Cynthia wines, and polymeric pigment increased as wines aged (Main and Morris 2007), so monoglucosides may determine the color stability and aging potential of these wines. Continued research is necessary to determine which practices will impact the quality of wines produced from red hybrid grape cultivars.

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CHAPTER 4

FUTURE WORK

In order to increase the profitability and success of wineries in cold climate regions of the United States, it is imperative to investigate factors involved in developing and selling wines. Whether in the winery or the tasting room, there is much improvement that can be made to satisfy consumers and generate more purchases. In the winery, a greater understanding of the effects of certain winemaking techniques, particularly exogenous tannin addition, will help improve the quality of hybrid wines that are commonly produced in cool climate regions. In addition, further research specifically studying hybrid wines and winegrapes needs to be done to better understand and extract phenolic compounds. In the tasting room, looking deeper into the many attributes that affect consumer decision and enjoyment will help enhance the tasting room experience.

Future work involving the effect of tannin addition on the phenolic concentration of hybrid wines may be slightly guided by this work. As later tannin addition showed possible effects on increasing phenolic compounds, it would be interesting to sample these wines in the future to decide whether later tannin addition results in a lasting effect. In addition, these wines could be analyzed for polymeric color to determine whether tannin addition increases the color stability. Sensory work also needs to be done on these wines to determine whether exogenous tannin addition results in improved sensory characteristics and whether timing of addition affects these sensory characteristics. Another important aspect of this work was the idea of creating a BLEND wine that combines a wine with tannin added at crush but no skin contact with a wine that has seven-day skin contact but no tannin addition. However, in order to compare treatments, the concentration of exogenous tannin in the BLEND needs to be 400 ppm. Thus the tannin

dosage to the wine fermented without skin contact (assuming a 50:50 blend) would need to be increased to 800 ppm in order to compensate for the decrease in concentration when blended. Secondly, the ratio of macerated wine without tannin added to non-macerated wine with tannin added should be increased, as anthocyanin concentrations in the BLEND wine were much lower than other treatments, and copigmentation and polymeric color favor a large concentration of anthocyanins (Boulton 2001). The theory behind the BLEND treatment is to ameliorate any unpleasant sensory characteristics of the exogenous tannin addition during fermentation, as yeast improve wine flavor (Scharpf et al. 1986) and may reduce off-aromas during alcoholic fermentation (Darriet et al. 2002; Guerche, S.L et a. 2006), while avoiding the opportunity for solids to bind the tannin during maceration (Cerpa-Calderón and Kennedy 2008).

More research should also be done to better identify tasting room factors in boutique wineries that influence consumer choice and consumer purchase decision. It would be beneficial to determine the effect of only removing subjective descriptors, as these descriptors are dependent on consumer sensory opinion. It would also be interesting to assess the impact of a tasting sheet with a predetermined flight versus a tasting sheet that allows complete consumer choice. With so many factors that affect the tasting room experience, it is important to continue investigating the elements that will bring more profitability to boutique wineries.

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